GENETIC AND PHYSIOLOGICAL STUDIES RELATING APPETITE, LEAN GROWTH AND FATNESS TO REPRODUCTIVE PERFORMANCE IN MICE

BY

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CONTENTS

ABSTRACT

An improved understanding of the genetic and physiological relationships between reproduction and growth in mammals has been sought by studying lines of mice selected for one of three criteria, either appetite (A), fat percentage (F) or total lean mass (P). For each criterion lines were selected for high (H) or low (L) performance, with unselected controls (C). In the A and P lines, first litter size changed in the direction of the selected criterion, the changes being larger and more rapidly established in the A than in the P lines. At generation 10, the differences in litter size between high and low lines were 2.6 and 1.0 live young born in the A and P lines, respectively. Changes in ovulation rate were the primary reason for changes in litter size, the differences between the high and low lines being 3.8 and 3.1 corpora lutea for the A and P lines, respectively. Associated changes in body weight could explain the alterations in ovulation rate and live foetus number (putative litter size) in the P lines, but not those in the A lines. There were no consistent differences in first litter size or ovulation rate in the F lines.

The observations at first litter in the A lines could also not be explained by a change of age at which mice displayed a peak in reproductive performance, since the correlated responses in litter size and ovulation rate to all three selected criteria were not found to be age or parity dependent. In addition, fertility and survival of breeding females to 7.5 months of age were not altered by previous selection history in either the A, F or P lines.

To further explore the physiological basis of the changes in litter size in the A lines, the activity of prolactin was suppressed in females with injections of bromocryptine. The results suggested that prolactin does not play a role in mediating relationships between appetite and ovulation rate in the mouse. In conclusion - the large changes in litter size in the A lines can be explained in

genetic terms by selection accumulating pleiotropic genes with relatively small effects on appetite and ovulation rate.

- Ovulation rate and litter size are positively correlated genetically with lean mass, but not with fat percentage in mice.

- Fertility is not genetically correlated with appetite, fat percentage or lean mass in mice when they are interval-bred for a major portion of reproductive lifespan.

The results of the thesis suggest that:

(i) selection for increased lean growth in commercially bred animals will probably lead to increases rather than decreases in litter size;

(ii) selection against fatness in pigs will probably not lead to a decline in reproductive performance;

(iii) infertility problems in sheep, cattle and humans are probably not strongly correlated genetically with fatness;

(iv) Alternative selection criteria for improving reproductive merit in farm animals might be found by exploring relationships between appetite, metabolic rate and reproductive performance.

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ABBREVIATIONS

ACTH	adrenocorticotrophic hormone
ALLTOT	number of young born alive or dead, summed over parities.
A.L.S.	number of young born alive at a particular parity.
ALVTOT	number of young born alive, summed over parities
BIRTH.WT.	weight of the whole litter at birth (alive and dead young)
BW	body weight
BSA	bovine serum albumin
CB154	bromocryptine, (2-bromo-α-erogcryptine mesilate)
^{o}C	degrees of centigrade
C	control
cpm	counts per minute
CHS	cysteamine (2-mercaptoethylamine)
d.f.	degrees of freedom
EDTA	ethene diamine tetra-acetic acid
e.g.	for example
FI	food intake
FSH	follicle stimulating hormone
GFPW	gonadal fat pad weight
GnRH	gonadotrophin-releasing hormone
g	gram
GH	growth hormone
h^2	heritability
H	high
hr	hour

I.M.	number of implants
^{125}I	radioactive isotope of iodine
I^{131}	radioactive isotope of iodine
i.e.	that is
L.F.	number of live foetuses
mCi	milli curie
mg	milligram
ml	millilitre
min	minute
M	molarity
mPRL	mouse prolactin
mRNA	messenger ribonucleic acid
ng	nanogram
NIADDK	National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases.
$NaHCO_3$	sodium bicarbonate
LH	luteinizing hormone
L	low
O.R.	ovulation rate
P.B.I.	pairing to birth interval
P1	parity 1
P2	parity 2
P3	parity 3
P4	parity 4
PBS	phosphate-buffered saline
POS.	post-implantation survival
PRE.	pre-implantation survival
P.S.	pre-natal survival

PRL	prolactin
r_g	genetic correlation
r_p	phenotypic correlation
rpm	revolutions per minute
SPF	specific pathogen free
S.E.	standard error
TSH	thyroid-stimulating hormone
T_4	thyroxine
T.L.S.	total number of young born, alive or dead, at a particular parity
T_3	triiodothyronine
12 DY.WT.	twelve day weight of a whole litter
21 DY.WT.	twenty-one day weight of a whole litter
uCi	micro curie
ug	microgram
ul	microlitre
vs	versus
wt	weight
yrs	years

GENERAL INTRODUCTION

An understanding of the relationships between appetite, growth, carcass composition and reproductive performance in mammals is relevant to the fields of animal breeding, human genetics and the development of evolutionary theory. Firstly, in many systems of animal production, the rate and quality of animal growth, the amount of food eaten and the number of offspring produced by each parent are all major factors in determining costs and returns. A breeder therefore is in a better situation to select profitable animals if he or she knows the relationships which exist between these traits. Secondly, the treatment of cases of human obesity could be based on more objective information if it were known, for instance, whether child-bearing ability or longevity was genetically associated with fat percentage of the body. Thirdly, a knowledge of relationships between appetite, general metabolism and reproductive performance, for example, may give valuable clues to understanding the process of speciation. Developing this knowledge in each of the species concerned is not practicable for cost, time and ethical reasons. The use of laboratory species offers an alternative approach, for several reasons. Firstly, they have a low unit cost and have short generation intervals and secondly, the results of experiments with laboratory animals should be relevant to other mammalian species, provided the information obtained is interpretated at the level of basic physiology or genetic mechanisms, rather than for directly extrapolating to the species of interest.

In this thesis, results of studies with mice are reported. Where appropriate, the results are used to draw inferences about larger mammalian species, within the limitations mentioned above. An experiment in which lines of mice have been selected for growth and fatness at Edinburgh University (Sharp, Hill and Robertson, 1984) presented an opportunity to examine the genetic and physiological relationships between the selected traits and reproductive

performance.

As a number of topics have been dealt with in the experimental sections, to aid the reader, a brief account of the contents and rationale of the thesis is given below. More detailed accounts of the reasons behind the research are given at the start of each experimental section.

Section 1 reviews the literature for evidence of genetic relationships between growth and reproduction in mammals and discusses what physiological mechanisms might be involved in mediating such connections.

Female reproductive performance at first litter is reported in Section 2 for mice selected for ten generations for one of three criteria: either appetite, fat percentage or total lean mass. An examination of the major components of litter size, namely ovulation rate and pre-natal survival is also reported for these lines, to observe which component had been affected most by the selection practiced.

An examination of four parities of the same selection lines of mice is reported in Section 3. This study was conducted primarily to test whether the differences in litter size found among the lines at first litter, were present at subsequent litters or, were merely reflections of changes in the time of life at which mice of the differing lines showed a peak in reproductive performance. Given the length of study required, an account of the effects of selection on survival characteristics of the mice is also given.

In Section 4, an experiment is described which examines the role of the hormone prolactin in helping to mediate relationships observed between appetite and ovulation rate in the mouse; these relationships are reported in Section 2 and 3.

Section 5 reports the methodology and validation of a radioimmunoassay for measuring prolactin. This assay was a necessary adjunct to the experiment reported in Section 4.

Finally, Section 6 is a general discussion of the findings of the thesis in the context of genetic and physiological relationships of growth and reproductive performance in mice and in other mammals.

1. LITERATURE REVIEW - GENETIC AND PHYSIOLOGICAL RELATIONSHIPS BETWEEN GROWTH AND REPRODUCTION IN MAMMALS

1.1. INTRODUCTION

The inheritance of differences in performance of growth and reproduction in mammals has been shown in general to be quantitative in nature, involving many genes, each of small effects. That the two traits may bear a degree of genetic control in common should not be too surprising given their complexity and the possibility of overlapping biochemical pathways.

As background information for the experimental sections of this thesis, the following review examines evidence for genetic relationships between growth and reproduction in mammals, the emphasis being on female performance. Many of the citations are of research with laboratory animals and, where appropriate, this information is used to draw conclusions about the relationships in mammals in general. Rather than leaving the review with an entirely genetic framework, an effort has also been made to briefly examine what is known about the physiological mechanisms involved in mediating relationships between growth and reproduction and to suggest some possibilities for further examination.

Before proceeding, definitions of growth and reproduction are needed as the scientific literature deals with a variety of aspects of the two traits, sometimes without sufficient clarification.

Definitions

Growth - 'A change in the body of an organism, and in the cells comprising it, accompanied by cell division, by the utilisation of material, and, nearly always, by increase in the size and weight of the organism or of the part under consideration. Once growth has occurred, its results cannot be reversed'. (Chambers' Technical

Dictionary). Growth in mammals therefore coincides with a large proportion of their life, from conception through an accelerating growth phase into a decelerating phase, when it eventually reaches a dynamic equilibrium in respect of size and weight (defined as 'maturity'), after which further increases are insignificant. In this review, growth as a trait is discussed as either body size, often measured indirectly as the weight of an animal, or as gain (growth rate), defined here as the increase in weight of the body per unit time.

Reproduction - 'The process of generation of new individuals whereby the species is perpetuated'. (Chambers' Technical Dictionary). This process of course can occur numerous times throughout a mammal's life. Female reproductive rate, the total number of offspring born to each dam, can be divided into the following components:

i) The number of litterings. This is dependent upon age at puberty, inter-breeding intervals and reproductive lifespan.

ii) The performance at each littering.

At each littering, reproductive rate can be divided further into:

- % fertility (the proportion of females giving birth of those exposed to males).
- prolificacy (litter size of those females giving birth).

Prolificacy itself has various components :-

- ovulation rate (number of ovulations per individual ovulating).
- fertilization rate (successful conceptions of those individuals mated or those ova exposed to spermatozoa), and
- pre-natal survival (embryos surviving to term of those successfully conceived).

Ovulation and pre-natal survival rates contribute much more to variation in litter size than fertilization rate in mice (Bateman, 1958; Bradford, 1979), cattle, sheep and pigs (Hanrahan, 1982). In addition, paternal effects are likely to account for only a very small proportion of the variation in reproductive performance in these species (Hanrahan, 1982), although Finn (1964) did find a small but significant effect of individual male mice on the size of the litter they sired. Fertilization rate and paternal effects will not be discussed further in any great detail.

Ideally, information about relationships of growth with age of puberty and with inter-breeding intervals could have been included, but has been excluded as it is not dealt with in the experimental sections of the thesis.

1.2. GENETIC AND PHENOTYPIC RELATIONSHIPS BETWEEN GROWTH AND REPRODUCTIVE PERFORMANCE

1.2.1. General

Much of the information concerning relationships between growth and reproduction in mammals is phenotypic, which includes both genetic and environmental components, the relative magnitude of each being unknown unless further efforts are made to estimate them. As such, phenotypic relationships give only a preliminary indication of the genetic connection. Breed or 'type' comparisons can provide useful genetic information, but it is difficult to quantify the magnitude of a genetic correlation from this type of data. Hill (1985) argues that associations between traits like body size and reproductive performance may not be the same across breeds as those within, as in the evolution of distinct populations (breeds being an example), natural or artificial selection or both have probably been involved.

Inbred strains, particularly of laboratory animals, have been used extensively in many fields of science and thus information on their growth and reproductive characteristics is available for examination. Although there can be large genetic differences between inbred strains in growth and reproductive performance, the fact that they are derived from an unknown combination of natural selection, artificial selection and 'drift' (Sharp, Hill, and Robertson, 1984) makes it difficult to determine the size of the genetic association.

More reliable information can be obtained from selection experiments in which components of growth or reproductive performance or both have been under selection. Caution, however, is also needed in interpreting the results of selection experiments especially where effective population size is small (Falconer, 1973; Eisen, Hanrahan, and Legates, 1973).

1.2.2. Phenotypic and between-breed or type data

Using a number of breeds of rabbits and crosses between them, Gregory (1932) found that ovulation rate and litter size appeared to be functions of the body weight of the doe, the heavier breeds having higher ovulation rates and litter sizes than the lighter breeds. Hammond's (1934) report is in agreement.

In dairy cattle, twin frequency decreased in general with lessening body size from Holsteins to Guernseys, Ayrshires and Jerseys (Lush,1925). Similarly, in the dog, Kaiser (1971) reports from information obtained from official statistics and replies to questionnaires that the size of the litter in the breeds investigated is in direct proportion to the size of the parent (height at the withers).

These essentially between-breed or type comparisons have also been made in sheep. A weak association was found between lambing

performance and body size among three hill breeds kept in the one location, the Welsh breed, being the smallest also had the lowest number of lambs born per ewe (Wiener, 1967). Positive associations between various measures of litter size at later ages and live weight either at the yearling stage (Columbia, Corriedale and Rambouillet breeds in America; Terrill and Stoehr, 1943) or 6 months of age (Scottish Blackface; Purser and Roberts, 1959) have been recorded. In addition, positive phenotypic correlations (r_p) between yearling live weight and the total number of lambs born per ewe have been reported for the Australian Merino (Young, Turner and Dolling, 1963, r_p = Ø.12, over the ewe's first three lambings, and Kennedy, 1967 r_p = Ø.Ø7, first lambing), the American Rambouillet (Shelton and Menzies, 1968; r_p = Ø.13, for 'lifetime' of the ewe) and the New Zealand Romney (Ch'ang and Rae, 1972; r_p = Ø.23, over the ewe's first three lambings).

A number of investigators have also examined the correlation between live weight, live-weight change and ovulation rate in the ewe. In general, where a wide range in both ovulation rates and live weights existed at the time of mating, a strong positive correlation was evident (see references in Cumming, 1977; Morley et al., 1978).

Although experiences with laboratory mice may be somewhat different, Batten and Berry (1967) did not find a consistent association between maternal size and litter size in mice caught in the wild; mice caught on farms showed a positive correlation, but there was virtually none in mice caught under island conditions, where they suggested some other factor could be limiting litter size. In the more predictable environment of the laboratory, a positive correlation between maternal weight at time of mating and subsequent litter size has been demonstrated in mice, for instance, by studying control strains in selection experiments, like Falconer's JC strain (Falconer, 1967a). The work of MacDowell, Allen and MacDowell (1929) examined these relationships in mice in terms

of ovulation rate. They found a positive correlation between mouse weight at conception and counts of corpora lutea. Similar phenotypic relationships have been reported in mice where ovulation rate has been measured directly (Land, 1970).

Turning to the human, MacArthur (1942), in reviewing the work of others suggests that stature is related to the incidence of twins and multiple births in European peoples; the incidence being highest among Nordic peoples , who have the largest stature, and lowest among the smaller statured people from Mediterranean areas. While acknowledging the difficulties of interpreting the European data, which was based on birth statistics, MacArthur also claims that the association between body size and twinning apparently holds in the black and yellow races as well as in the white race, emphasising that the small-structured Japanese have a very low fraternal twinning rate, whereas the large-structured American negro has the highest.

Several workers have reported a positive relationship between size or weight in pigs and litter size, using comparisons between strains of the Poland China breed which vary in size (Hetzer and Brier, 1940; Zeller, 1940), or a variety of breeds and crosses (MacArthur, 1942; Stewart, 1945; Omtvedt, Stanislaw and Whatley, 1965). However, the relationships observed between the various measures of growth used and litter size were not strong (Young et al., 1978). Measures of growth in the female pig are positively correlated with ovulation rate (Squiers et al., 1952; Rathnasabapathy, Lasley and Mayer, 1956; Reddy, Lasley and Mayer, 1958; Young and Omtvedt, 1973; Young, Omtvedt and Johnson, 1974; Young, Johnson and Omtvedt, 1977a,b; Young et al., 1978), however the correlation between measures of growth and litter size (or embryo counts during pregnancy) is generally weaker (Rathnasabapathy et al., 1956; Omtvedt et al., 1965; Young et al., 1974; Young et al., 1977a,b; Young et al., 1978).

The general association between body size and number of progeny in domesticated and laboratory animals holds equally for wild mammals (and birds) in nature (MacArthur, 1942), and reveals interrelations between several of the so-called 'climates rules' (Rensch, 1939). In a wide ranging species of mammal or bird, the geographic races living in the colder parts of its range (e.g. in Northern latitudes or on mountain heights) tend in general to be larger in body measurements than the races of the same species inhabiting warmer regions (Bergman's Rule). It is now possible to relate this rule with two others, which were formulated by Rensch(1939) quite independently of it: (1) In European and North American faunal surveys the more Northern or alpine races of a mammalian species tend to have larger litters (the 'litter rule'); and (2) similarly, the Northern races of birds tend to lay more eggs in a clutch (the "egg number rule"). The two latter rules become in a sense mere corollaries of Bergman's Rule, since, on the view presented, body size and litter or clutch size are intimately correlated (MacArthur, 1942).

The information presented in this section obviously supports the view that there is a positive genetic association between body size and litter size in mammals. More critical evidence is presented in the next section, where attempts have been made to isolate genetic causes of correlation from environmental effects.

1.2.3. Estimates of genetic relationships in mice, pigs and sheep

Whereas the previous section examined information from numerous mammalian species, this section is restricted to reports from work with mice, sheep and pigs, because critical studies exploring genetic relationships between growth and reproduction have not been conducted to any great extent in other mammalia.

In genetic studies, it is important to distinguish between spurious associations of characters (as in the case of drift and

chance fixation of genes) and real ones. Pleiotropy is the main genetic cause of enduring correlations between characters whereas linkage is a cause of transient correlation, particularly in populations derived from crosses between divergent strains (Falconer, 1981). A correlation arising from pleiotropy is the overall effect of all the segregating genes that influence the two characters under consideration.

Genetic relationships between growth and reproductive performance have often been described by estimating the genetic correlation, which is the correlation of breeding values. Estimates of genetic correlations can be made by using information from parent-offspring relationships, covariance of sibs, and by using results from selection experiments. In many reports, estimates of genetic correlations suffer from poor precision, because of the relatively small size of the data sets. In these cases the sign of the estimates made and the direction of the correlated responses to selection are viewed together with those from similar studies instead of placing emphasis on the magnitude of the estimates of genetic correlations themselves.

Mice - Growth and first litter size

Tables 1.1 and 1.2 summarise the correlated responses obtained in reproduction where selection has been successfully practised on body weight and gain, respectively. Three reports are special cases where either traits strongly correlated to body weight and gain have been the selection criteria (Eisen, Legates and Robinson, 1970; McLellan and Frahm, 1973) or gain has been selected jointly with litter size (Doolittle, Wilson and Hulbert, 1972).

In general terms, litter size at first parity has changed in the direction of selection when growth has been the selection criterion (see references listed in Tables 1.1 and 1.2).An exception is a study by Bradford (1971). However, it appears that when litter size

TABLE 1.1. Summary of correlated responses in reproductive traits in selection experiments for body weight in mice.

Selection Criterion	Type of Selection	Effective Population Size	Duration (gens)	High-Low Correlated Response	Estimated genetic correlation	Reference
High (H) & low (L) 60 day wt	Progeny test & mass	16 to 31	8	+4.2 young born[b]	-	MacArthur (1944)
Same experiment	" "	" "	11	+6.1 corpora lutea	-	" "
" "	" "	" "	12 & 13	+6.9 corpora lutea	-	MacArthur (1949)
" "	" "	" "	22	+4.5 young born	-	" "
H, L 42 day wt	Within family	32	11	+4.2[a] live young born	-	Falconer (1953)
Same experiment	" "	"	H 34-40 L 25-31	+6.4 ova	-	Fowler & Edwards (1960)
H, L 42 day wt	Within family	40	40	+3.5[c] live & dead young born +2.6 live young born	-	White, Legates & Eisen (1968)
Same experiment	" "	40	35	+4.1 corpora lutea	-	Elliot, Legates & Ulberg (1968)
H, 12 day litter wt (standardised to 6 pups)	Within family	4[d] x 71	10	+0.4 live young born (High-Control diff.)	0.19	Eisen, Legates & Robison (1970)
H, L 84 day hind limb muscle wt	Retrospective mass	64	7	+1.7 live young at 3 days of age	-	McLellan & Frahm (1973)
H, L 42 day wt	Within family	6 x 32	23	+3.5 live young born	-	Falconer (1973)
H, L 56 day wt	Mass	32	14	+4.1 young born	-	Bakker (1974)
H, individual 21 wt in standardised litter	Within family	3 x 40	14	+1.7 young born (High-Control diff.)	-	Frahm & Brown (1975)
H 42 day wt	Mass	49	12		0.52±0.10	Eisen (1978)
H litter size	"	52	"		0.52±0.13	
H 42 day wt: L litter size	"	54	"		0.63±0.14 (Realised correlation)	
L 42 day wt: H litter size	"	49	"			
H, 21 day wt	Within family	37	32	approx.+2.5 young born	-	Baker, Cox and Carter (1984)
H, 42 day wt	" "	32	37	approx.+1.0 young born (High-Control diff.)	-	" " " " "
H, L 56 day wt	Within family	2 x 32	18(22)	+7.7[e] young born	-	von Butler et al. (1984)
H, L 56 day wt	" "	2 x 32	18(22)	+5.1 young born	-	" " "
H, L 56 day wt	Mass	2 x 16	18(22)	+11.0 young born	-	" " "
H, L 56 day wt	"	2 x 16	18(22)	+8.4 young born	-	" " "

a Correlated responses calculated on data from first and second litter.

b Young born - litter size unspecified as being live births or all births. Live young born - litter size specified as live births.

c Higher selection intensities were practiced in these lines.

d Number of replicates, unreplicated lines shown simply as effective population size, with no multiplication factor.

e Figures are at generation 22, from a personal communication.

TABLE 1.2. Summary of correlated responses in reproductive traits in selection experiments for gain in mice

(See the bottom of Table 1.1 for footnotes)

Selection Criterion	Type of Selection	Effective Population Size	Duration (gen)	High-Control Correlated Response	Estimated Genetic Correlation	Reference
High (H), low (L) 21 to 42 day wt. gain	Within family	24	H 16-20 L 16-19	+ 6.2 ova (H-L diff.)	-	Fowler & Edwards (1960)
H 21 to 42 day wt gain	Mass	30	29	+ 2.9 live young at[b] weaning	0.89	Rahnefeld et al. (1966)
H 21 to 42 day wt. gain	Mass	48	24	zero young born	-	Bradford (1971)
Same experiment	"	"	17	+3.8 corpora lutea		
H, 21 to 42 day gain and H litter size	Tandem, mass	2 x 98	8	no sensible way of presenting data	Rep I 0.60 Rep II 0.65	Doolittle, Wilson & Hulbert (1972)
H 21 to 42 day wt gain	Within family	2 x 62	14	+1.7 live young born	-	Eisen, Hanrahan & Legates (1973)
	" "	2 x 62[c]		+3.2 live young born		
H 21 to 42 day wt gain	Mass	2 x 74	8	Rep I + 2.1 young born Rep 2 + 1.0 young born	0.82 0.25	Wilson (1973)
H 21 to 42 day wt gain	Mass	4 x 60	12	+0.72 live young born	-	La Salle, White & Vinson (1974)
H 21 to 42 day wt gain	Mass	3 x 27	14	+2.0 young born	-	Frahm & Brown (1975)
H 21 to 42 day wt gain	Within family	32	37	Approx. +3.0 young born	-	Baker, Cox & Carter (1984)

is examined in terms of its major components, ovulation rate and pre-natal survival, a clearer picture emerges. Where reported, ovulation rate has changed in the direction of selection for growth, but pre-natal survival rate invariably declines (Fowler and Edwards, 1960; Elliot, Legates and Ulberg, 1968; Land, 1970; Bradford, 1971 and Barria and Bradford, 1981), which reduces the responses observed in litter size to well below their potential, the number of eggs ovulated.

Further evidence is available from experiments where either litter size or ovulation rate have been successfully selected for and body weights or gains have been measured as correlated responses. Body weights recorded at 6 weeks of age and at the time of counting eggs changed in the direction of selection for ovulation rate in primiparous females (Land, 1970). It appears that in selection experiments on litter size where maternal effects are small either because within family selection has been used, litters had been standardized or females had been mated at a relatively mature age, correlated responses in post-weaning growth have been in the same direction as the direct responses (Joakimsen and Baker, 1977, and Eisen, 1978). Bateman's (1966) report is in agreement, although no control was maintained to help determine symmetry of response. Earlier, Falconer (1965) had mentioned that 6-week weights in his selection lines had diverged in the same direction as direct response for litter size, once account had been taken of maternal effects. Likewise, maternal effects may have reduced the positive correlated responses observed in 8-week weights which only appeared after 10 generations of successful selection for increased litter size (Bakker, Wallinga and Politiek, 1978).

Genetic correlations. By the use of a double selection experiment, Eisen (1978) found that his 'realized' genetic correlation between 6-week weight and litter size of +0.63 agreed closely with a base population estimate; Doolittle, Wilson and Hulbert's (1972)

correlations between 3 to 6 week gain and litter size are similar in sign and magnitude, but the precision of the estimates are not given (see Tables 1.1 and 1.2 for figures).

Although Rahnefeld, Boylan and Comstock's (1962) and Rahnefeld et al.'s (1966) estimates of genetic correlations between gain and litter size from the same experiment vary widely (+Ø.15 and +Ø.89, respectively), they are at least both positive and probably not incompatible in magnitude considering the poor estimation of the various population parameters, and the possibility of correlations changing during the course of selection. Wilson's (1973) figures probably do not suffer as much from poor precision, because of larger effective population sizes of the selection lines, but variability between replicates is quite obvious, although all estimates are positive (estimates were +Ø.82 for replicate 1 and +Ø.25 for replicate 2).

Moderate to high positive genetic correlations between body weights (42 and 56 days of age), gain (21 to 42 days) and litter size at birth have been estimated with good precision from a random-bred population of mice (Hanrahan and Eisen, 1974). The figures were Ø.36, Ø.34 and Ø.58, respectively. Eisen et al. (1973) have reported a positive correlated response for litter size when selection was practised for post-weaning gain in lines derived from the same base population used for estimates made by Hanrahan and Eisen (1974).

Among the selection experiments for litter size, Joakimsen and Baker (1977) estimate realized genetic correlations of Ø.32 and Ø.62 between total number of mice born and 3 and 6-week weights, respectively. These figures, calculated from the divergence of high and low lines, are relatively free of complicating maternal effects, as the correlated responses in weights used were measured in cross-fostering studies. They suggest that the positive genetic correlations found in their experiment are most likely due to a

positive association between body weight and ovulation rate, while embryo losses and body weight were probably not correlated genetically. This is supported by the more direct evidence of Land and Falconer (1969) and Land (1970) regarding ovulation rate and the negative results of Bradford (1971) in failing to obtain correlated responses in litter size, even though ovulation rates had changed in the direction of selection for post-weaning gain (Bradford, 1971 and Barria and Bradford, 1981). Land (1970) concludes from his estimates that the genetic correlation between body weight and natural ovulation rate is positive and is probably greater than 0.4.

In summary, remembering that this section has concentrated on first litter size data as a measure of reproductive rate, there appears to be overwhelming evidence for a moderate to strong positive genetic correlation between growth and litter size in mice, the connection being primarily through ovulation rate.

Mice - growth, litter size at later parities and infertility

In most of the studies already cited, litter size has been thought of in narrow terms, as the performance at first litter. In most instances, the mice have given birth to their first litters by 12 weeks of age, with ovulation rate having been determined some 3 weeks beforehand. The female mouse is still actively growing at this time, albeit at a slower rate than in the early post-weaning period and its reproductive lifespan can last until 10 months of age or more (Roberts, 1961). It is therefore obvious that first litter performance represents a small part of the potential reproductive output of a mouse.

A number of workers have studied litter size in successive pregnancies of mice from lines previously selected for body weight and the general finding is that in high lines, lifetime reproductive rate is reduced, primarily through a shortened reproductive lifespan (Roberts, 1961; Nagai, Harris and McAllister,

198Ø, and von Butler et al., 1984). In these studies, mice from lines selected for high body weight had fewer, but in general larger litters than mice from lines selected for low body weight or from unselected control populations. In other words, while they were still reproductively active, mice from high body weight lines retained at least some of the advantage in litter size widely reported at first litter (see previous section).

Infertility can occur at an early age in mice lines selected for high body weight or gain (Falconer, 1955; Fowler and Edwards, 196Ø; Roberts, 1967; Bradford, 1971; Eisen et al., 1973; Falconer, 1973; Bakker, 1974; La Salle et al., 1974; Frahm and Brown, 1975; Barria and Bradford, 1981 and von Butler et al., 1984), but not in all cases (Elliot et al., 1968; Eisen, 1978; Baker et al., 1984 and von Butler et al., 1984). In addition, selection for low body weight has also increased infertility (Fowler and Edwards, 196Ø; Elliot et al., 1968; Falconer, 1973; Bakker, 1974 and von Butler et al., 1984) though not always (von Butler et al., 1984). Poor male libido has been implicated for the infertility problems in a high body weight line described by Falconer (1955), (Fowler and Edwards, 196Ø) and in a low body weight line described by White, Legates and Eisen (1968)(Elliot et al., 1968), and inbreeding effects may have contributed to the levels of infertility reported, especially where population sizes have been small. However, the decline in fertility in lines selected for body weight is too widespread amongst the literature to be brushed aside by arguing that male libido and inbreeding can explain all. For instance, Fowler and Edwards (196Ø) present evidence that a decline in fertility of a line of mice selected for low body weight was due to an increase in sterility among females.

It has been postulated that the decline in fitness (of which fertility must be a part) observed following directional selection for a metric trait (Lerner, 1954) may be due to the homeostatic model (Lerner, 1954) or the metric deviation model (Robertson, 1956). The homeostatic model assumes that the decline in fitness

as a correlated response to selection is due to increased homozygosity at loci affecting the quantitative trait, whereas the metric deviation model argues that extreme phenotypes are intrinsically less fit. Both these models allow the assumption that there is an optimum phenotype for body weight (or a character highly correlated genetically with body weight) and that deviations from this optimum lead to a decline in fitness (Eisen et al., 1973).

Mice lines previously selected for increased body weight or gain are prone to fatness, especially at older ages, and it has been argued that overfatness may decrease fertility (see reviews by Roberts, 1965, 1979). Roberts (1974) cites one instance where infertility problems were overcome by mating females one week earlier than usual, before fat accumulated. It is also of interest to note that in the high body weight line described by White et al. (1968), neither fatness (Lang and Legates, 1969) nor infertility increased (Elliot et al., 1968); in contrast fatness and infertility are associated in a number of similar experiments (see Eisen et al., 1973 for discussion and references). However, more direct evidence on the influence of fatness per se on fertility and fitness in mice is lacking. Such evidence is needed before definite conclusions can be drawn about the genetic relationship between measures of growth and infertility in the mouse.

Pigs

Information regarding genetic relationships between growth and reproductive performance in swine is available from analyses of records from central testing stations and recording schemes as well as from results of selection experiments. Johansson (1981) has conveniently summarised estimates made in the literature of the genetic relationships of fertility with daily gain, feed conversion and carcass traits; a modified version of which can be seen in Table 1.3.

TABLE 1.3. Genetic correlations between traits of the growing pig and litter size, estimated from testing station and recording scheme data.

Litter size (Number of live piglets)	Country of study	Breed	No.of sows	Traits of the growing pig: Daily Gain	Length of carcass	Killing out	Backfat	Hindquarters %	Ham & Loin %	Reference
Litter size at birth. 1st to 4th litters	U.S.A.	Various	-	0.06[a]						Vogt,Comstock & & Rempel (1963)
Litter size at birth. 1st to 3rd litters	France	Large white	1140	-0.08	-0.33	-	0.11	-	.02	) Legault (1971)[b]
Litter size at weaning (8wks)		"	1140	0.09	-0.39	-	0.08	-	.18	)
Litter size at birth[g]	Britain	Large white								
-1st litter			2175	-0.17(0.16)[e]	-	-0.11	-0.47[f] to-0.08	-0.33	-	)
-2nd litter		"	1146	0.15(-0.02)	-	-1.08	-0.42 to-0.03	-0.64	-	) Morris (1975)[c]
Litter size at birth		Landrace								)
-1st litter			1321	0.42(-0.15)	-	-0.20	-0.48 to-0.03	-0.14	-	)
-2nd litter			653	1.25(0.44)	-	-0.67	-1.84 to-0.22	0.03	-	)
Litter size at 3 weeks		Large white								)
-1st litter			2175	-0.08(0.19)	-	-0.45	-0.53 to 0.10	-0.39	-	)
-2nd litter			1146	0.38(-0.20)	-	-0.88	-0.31 to-0.06	-0.26	-	)
Litter size at 3 weeks		Landrace								)
-1st litter			1321	-0.02(-0.19)	-	-0.14	-0.02 to 0.23	0.06	-	)
-2nd litter			653	0.68(1.12)	-	-0.93	-1.12 to-0.11	-0.43	-	)
Litter size at weaning 1st & 2nd litters	Denmark		13600	-0.12	-		-0.04			Christensen (1980)[d]
Litter size at 3 weeks	Sweden	-								
-1st litter			3875	0.01	-	-	0.18			) Johannson
-2nd litter			3875	0.59	-	-	0.04			) (1981)[d]
Litter size at birth	Sweden									
-1st litter		Swedish	7275	-	-	-	0.13			)
-2nd litter		Landrace		-	-	-	-0.05			)
Litter size at birth										) Johannson & Kennedy (1983)[d]
-1st litter		Yorkshire	3797	-	-	-	0.22			)
-2nd litter				-	-	-	0.13			)

a. Correlation not significant
b. Standard errors (SE) not given; correlation of litter size at 8 weeks, ham and loin percentage significant ($P < 0.05$); remainder non-significant
c. SE's 0.30 for Large White, 0.45 for Landrace.
d. SE's not given.
e. Estimates in brackets are based on testing of boars, otherwise the estimates are from testing of sibs (castrate male and gilt pair)
f. Measurements taken from several sites.
g. Litter size not specified as being the number of piglets born alive or the total number born.

Genetic correlations of daily gain and first litter size appear to be near zero, but those with second litter are positive. In two reports gilt litter size and her litter size at second parity appear to be only moderately correlated (Johannson, 1981; Legault, 1983); Johannson (1981) quotes a figure of Ø.41 ± Ø.14 for the number of piglets born alive, and implies that the gilts' reproductive performance may not bear highly similar relationships to growth traits as a female on her second or subsequent litters. However, there is an obvious need for more research on this point. For instance, gilts mated during the first fertile oestrous cycle following puberty may have lower litter sizes than gilts mated on the second, third or fourth cycle and this could conceivably affect the magnitude of the genetic correlation of litter size of gilt litter with that of later parities. Estimates of genetic correlations approaching unity between gilt litter size and litter size at subsequent parities have been made recently where gilts have been mated on the 3rd or 4th oestrous cycle following puberty (E. Avalos, personal communication).

It is unfortunate that first and second litter information is confounded in Christensen's (1980) large study, as the untangling of the two might have helped to explain the negative estimate of the correlation between litter size at weaning and daily gain. Furthermore, testing procedures do vary between countries, which may thus lead to differing relationships between traits measured in the test and possibly in turn, their relationships with reproductive traits, so caution is required in interpreting lack of agreement between correlation estimates (Johannson, 1981).

Amongst the carcass traits, killing out percentage has a uniformly negative correlation (undesirable) with litter size, although the estimates are based entirely on the British study of Morris (1975). Backfat depth appears to have a small favourable correlation with litter size in the studies of Morris (1975) and

Christensen (1980) but again, difficulties emerge as few of the carcass measurements are likely to be identical between the different studies (Johannson, 1981); other studies quoted in Table 3 are in disagreement with the sign of the genetic correlation. Carcass length in the French study (Legault, 1971) appears to have a moderately unfavourable correlation with litter size.

In an effort to group together the various measures of growth, and of carcass characteristics used in his study, Legault (1971) used canonical correlation coefficients to show that litter size was practically independent from growth and from carcass characteristics. Although the size of estimates of genetic correlations of litter size with growth and carcass traits are small, viewing all the data presented in Table 1.3 leads one to disagree with Legault's conclusions. The correlation structure of the results obtained by Morris (1975) is reasonably consistent, and he offers the following explanation for a correlation between the measures of growth used and reproduction - 'Since the criterion for slaughter of bacon pigs was weight rather than age, then the carcasses of pigs which were more efficient during post-weaning growth were leaner, and by implication less mature at the slaughter weight. They were therefore the pigs with the potential for greater mature weight. A positive correlation between litter size and mature size would then explain the data observed here'. Hill and Webb (1982) agree with this explanation in a review paper.

It is difficult to draw definite conclusions from the pig data presented so far, however more information is available from selection experiments. If litter size is genetically correlated with growth and carcass characteristics in swine, directional changes should occur in the unselected trait(s) as an indirect consequence of selection. All this assumes of course that the selection experiments are sufficiently large to quantify responses and sound enough in their design to allow their proper analysis. Like numerous selection experiments with mice, those with pigs have

often been unreplicated with small effective population sizes (see Table 1.4). Where genetic correlations have been estimated, the precision has frequently been poor and it is more informative to examine the direction and size of the correlated responses, although even these can show marked variation in light of Falconer's (1973) findings on variability in correlated responses amongst small, replicated selection lines. It is also important to point out that in many of the selection experiments, the responses in litter size refer to gilt performance (Table 1.4); as already mentioned, this may not be representative of the same animals in subsequent parities.

In achieving success in selection for small size, Dettmers, Rempel and Comstock (1965) report no change in litter size. A number of genetic studies of backfat thickness or equivalent have been reported and where direct responses have been obtained to selection, correlated responses in litter size have been absent in some studies (Dillard, Robison and Legates, 1962; Gray et al., 1968) but present in others (Berruecos, Dillard and Robison, 1970; Hetzer and Miller, 1970). Berruecos et al. (1970) found a significant decline in litter size during 5 generations of selection for low backfat thickness, whereas Hetzer and Miller (1970) reported a confusing pattern, with Durocs showing a trend of increasing litter size with selection for high backfat and a decreasing one for selection on low backfat; with Yorkshires the reverse was the case. In neither the study on Durocs or Yorkshires were these trends statistically significant.

A number of other workers, selecting for a variety of traits, have mentioned reproductive performance; Fahmy and Bernard (1972) did not find any significant effects on litter size after 10 generations of selection for feed utilization, carcass score (includes measurements of carcass length, backfat thickness, loin eye area and a subjective belly grading) or for a combination of the two. Although Webb and King's (1976) selection criteria were not

TABLE 1.4. Summary of correlated responses in reproduction from selection experiments for growth and carcass traits in swine.

Selection criterion	Type of Selection	Effective Population size	No. of generations	Parity of litter size information	Correlated Response in litter size at birth.	Estimates of Genetic correlations	Reference
Low(L) 140 day wt	Mass	40-60	11	Mostly gilt	None	-	Dettmers, Rempel & Comstock (1965)
L backfat/Body wt ratio	Not specified probably mass	Selected line 25-40 control line 15-25	5	Probably gilt	Not given	[a] Litter size & 130 day wt = -0.30 Litter size & backfat, adjusted to 140 lb = +0.09 body wt.	Dillard, Robison & Legates (1962)
L backfat at standardised 79.4 kg body wt.	Mass, with restriction of no more than 1 boar from each litter	'Spring' 21-24 'Fall' 21-24	5	Probably gilt	None - also no changes in ovulation rate	-	Gray et al. (1968)
L back fat at 130 days, adjusted to constant 63.6 kg	Within family	Selected line 24. Control line 21	5	Gilt	Regression of litter size at birth[b] on generation number = -0.30±0.08 (P<0.01)	Not estimated	Berruecos, Dillard & Robison (1970)
High (H) & L backfat at an average 79.4 kg body wt.	Mass	Selected lines 16. Control line 24	Duroc 13 Yorkshire 11	Mostly gilt	Duroc (H fat-control = (-0.26 piglets at (birth[b] (L fat-control = (+1.56 piglets at (birth Yorkshire (H fat-control = (+0.66 piglets at (birth (L fat-control = (-0.55 piglets at (birth Trends not statistically significant (P>0.05)	'Realized' correlations. Litter size at birth[b] & backfat Duroc = -0.11 Yorkshire=+0.05	Hetzer & Miller (1970)
H feed utilization (Line 1) H carcass score (Line 2) Index of both (Line 3)	Within family for males - females not selected	16	10	Gilt	Line 2 had 0.44 and 0.34 more total piglets born (alive & dead) than Line 1 and 3 resp. (P>0.5)	Not estimated	Fahmy & Bernard (1972)
H Index (HP) (H growth rate, L fat) L Index (LP) (L growth rate, H fat)	Within family for boars, mass for females	Difficult to estimate from information given probably >35	10	Gilt, 2nd, 3rd and later litters	HP - Control =+0.37 live piglets born LP - Control =-0.93 live piglets born	Live piglets born & daily gain = -0.07 ±0.44 Live piglets & backfat =-0.21±0.33 Live piglets & index =0.19±0.33 'Realised' correlation of live piglets born & index =0.08±0.04 (P>0.05)	Vangen (1980)

a litter size - value not specified as to whether it refers to birth or older ages, nor whether it includes any stillborn piglets

b litter size at birth - value not specified as to whether it includes stillborn piglets.

identical and their experiment was open to immigration from a variety of breeds, they also report no evidence of a decline in gilt reproductive performance, except for teat number. They aimed to develop a synthetic pig sire line, by selecting initially on carcass length and backfat measurements, but after 6 generations the criterion was modified to an index combining growth rate, food conversion ratio and average ultrasonic backfat.

Findings such as Webb and King's (1976) have not been universal, especially where selection experiments have placed emphasis on growth rate. Fredeen, Mikami and Sather (1976) found predominantly increased litter size when selecting for 9 generations in three lines: maximum growth, minimum fat, and an index combining the two; the greatest response in litter size being found in the index line. Similarly in Vangen's (1980) high and low index lines of daily gain and backfat, litter size at birth changed in the direction of selection; the high line criterion being high daily gain and minimum backfat, the low line criterion being vice versa. As Vangen obtained several litters from each female, he was able to examine the effect of parity on litter size within each line, which was found to be highly significant. However, Vangen's analysis does not allow presentation of direct evidence for the effect of parity on correlated responses. This is unfortunate as such information would have provided an experimental check on the trends suggested in the studies summarized in Table 1.3.

One would expect further information on growth and reproductive relationships to be available where growth has been studied as a correlated response to selection for litter size. However, there have been very few reports of such selection. French workers initially achieved success in selecting for increased litter size (Ollivier, 1973) although in later generations of selection the positive response vanished (Ollivier and Bolet, 1981). No account of correlated responses in growth is available from the above study or in those reported by Rutledge (1980) and Bichard and Seidel

(1982).

Components of litter size have been directly selected for in swine. Direct responses indicate success in increasing ovulation rate by selection in gilts (Zimmerman and Cunningham, 1975), but there have been virtually no correlated responses for growth (Newton, Cunningham and Zimmerman, 1977), carcass traits (Newton et al., 1977; England et al., 1977) or litter size (Cunningham et al., 1979), although a more recent paper reports slight increases of litter size in subsequent generations of selection (Johnson, Zimmermann and Kittok, 1983). Selection is now underway by the same research group, based on an index of both ovulation rate and embryo survival, and, although early results of reproductive traits are promising (R.K. Johnson, personal communication) no information is as yet available on correlated responses in growth characteristics.

Finally, Young et al. (1977a,b) and Young et al. (1978) have used a number of methods in estimating genetic correlations between pre-breeding traits and reproductive traits. In Young et al.'s (1977a,b) case, the records of 339 purebred Duroc, Hampshire and Yorkshire gilts, and 192 two-breed cross gilts arising from matings among the three breeds were used; unfortunately negative estimates of sire variance precluded estimations of genetic correlations involving embryo number and litter size as traits. However, all the measures of growth taken had positive genetic correlations with ovulation rate, with the relationship being stronger for traits measured later in the growth phase than those measured earlier. In contrast, traits measured early in the growth phase (birth weight and weaning weight) were the only ones which had large genetic correlations (-Ø.9Ø and +Ø.91, respectively) with a measure of embryo survival, the number of corpora lutea per embryo (Young et al., 1977a). It should be emphasized here that the reproductive traits in the above work pertain to gilts, which may not be very representative of animals on second or later parities

(Johannson, 1981).

Using principal component analyses Young et al. (1977b) come to similar conclusions as in their earlier paper, in that there are some fairly large genetic correlations between growth measures and reproductive measures, but the phenotypic correlations are small due to large environmental correlations of opposite sign. Extending the principal component analysis approach to a larger data set of 2095 gilts, Young et al. (1978) report good agreement with Young et al. (1977b).

In summary, the pig information is somewhat inconclusive; where estimates have been made from family relationships, growth rate and litter size appear to be positively correlated genetically in sows rather than gilts, perhaps through a connection of growth rate with mature size. However, there are no clear trends evident from the various selection experiments that have been conducted, which could be as a result of gilt performance being the main criterion by which reproductive rate has been measured.

When litter size is examined in terms of ovulation rate and embryo survival, ovulation rate appears to be the trait which is positively correlated with measures of growth, in the case of estimates made from family relationships and principal component analysis. However, this evidence is not conclusive, as growth was unchanged in lines selected for ovulation rate (Newton et al., 1977). The literature is not clear as to whether backfat thickness is genetically correlated with litter size, in either a favourable or unfavourable direction, the confusion exists both among estimates made from family relationships and from among results of numerous selection experiments.

Sheep

In contrast to mice and pigs, the sheep is not a classic litter-

bearing species, although twins are quite common and some breeds are noted for incidence of litters of 3 or even more (Bradford, 1972). As such, the sheep represents an intermediate species between the monovular mammals such as the human, and the litter-bearers. Including it in this review broadens the scope of the discussion to represent relationships between mammalian growth and reproduction in general.

Most evidence comes from regression and variance and covariance analysis of records of animals in improvement schemes or experimental flocks (see Table 1.5). In regard to the size of study required to obtain precise estimates of genetic correlations, it is rather sobering to consider comments made by Forrest and Bichard (1974); 'where $h^2_1 = h^2_2 = r_g = \emptyset.2\emptyset$ (referring to heritabilities of trait 1 and trait 2, respectively, between which the correlation is being estimated) and the optimum design is assumed, a total of 16,000 individuals would need to be measured to adjudge the value of r_g to be significantly different from zero'. Despite the limited size of individual studies however, measures of growth, particularly body weight at the yearling stage appear in general to be positively correlated genetically with lambs born per ewe mated, and per ewe lambing (see references quoted in Table 1.5). It is important to note that the trait 'lambs born per ewe mated' is a combination of the ability to reproduce (fertility) and litter size (prolificacy).

Correlations of reproductive performance with weaning and pre-weaning weights are possibly biassed, as these weights are quite prone to maternal effects (e.g. Pattie, 1965a; Shelton and Menzies, 1968)(see Figures of Shelton and Menzies, 1968, and Eikje, 1975) and correction for these in the studies mentioned may not have completely removed their contribution towards environmental variation. The yearling figures are probably less biassed, given the likelihood of compensatory growth by twins or animals from higher order litters during the post-weaning period (Shelton and

TABLE 1.5 Summary of estimated genetic correlations between growth and reproductive traits in sheep.

Country of study	Breeds Involved	Size of Study	Reproductive Trait[c] (1)	Growth Trait (2)	Genetic correlation[a] between Traits 1 & 2	Reference
Australia	Merino	Approx.700 ewes between 55-77 sire groups	Lambs born/ewe mating - at first lambing (at 2 yrs of age) - at 3 yrs of age - at first 3 lambings	Body wt at 15-16 mths of age " " " "	+0.47) Not signifi-) cantly differ- +0.16) ent from zero +0.23) at the 5% level	Young, Turner & Dolling (1963)
Scotland	Scottish Blackface	2, 117 2 yr old ewes 343 sires	Lambs born/ewe lambing at 2,3,4,5 and 6 yrs of age	Body wt at mating	+0.44±0.11	Purser (1965)
Wales	Welsh	1, 474 2 yr old ewes 156 sires	Lambs born/ewe lambing at 2,3 and 4 yrs of age	Body wt at mating	+0.78±0.08	
Australia	Merino	Approx.650 ewes 58 sire groups	Lambs born/ewe mated to lamb at 2 yrs of age	Body wt at 15-16 mths of age	+0.20±0.38	Kennedy (1967)
U.S.A.	Rambouillet	Approx. 4500 lambing records	Lambs born/ewe mated to lamb at 3,4,5,6 and 7 yrs of age	Weaning wt Yearling wt	+0.06±0.05 +0.57±0.23	Shelton & Menzies (1968)
New Zealand	Romney	Approx.800 ewes.Approx. 40 sires	Lambs born/ewe mated - at first lambing - at second lambing - at first 3 lambings	Body wt at 14mths of age " " " "	+0.81 +0.56 +0.65	Chang & Rae (1972)
England	Clun Forest	Approx.1300 ewes. 132 sires.	Lambs born/ewe lambing Lambs born/ewe mated & present at lambing[b] (All lambings from 1 to 5 yr old ewes combined)	Body wt at mating " "	+0.21 -0.04	Forrest & Bichard (1974)
Norway	Dala, Rygja Cheviot, Spaelsana Steigar	40,000 ewes 6,723 sires	Lambs born/ewe lambing at 1,2,3 yrs or older[b]	'Spring' wt at 20-50 days of age Weaning wt	+0.04 -0.11	Eikje (1975)

a All estimates calculated from the sire component of covariance of half-sibs.

b Only studies containing records from ewes lambing at 1 year of age.

c In all cases litter size is not specified as the total born or the number born alive. In most studies, lambing is performed under extensive conditions, so the figures probably refer to the number of lambs born alive.

Menzies, 1968).

Where examined, the age or parity of the ewe does not appear to have a clear-cut effect on the magnitude of the genetic correlation (Young et al., 1963; Ch'ang and Rae, 1972; Forrest and Bichard, 1974), although the relatively large standard errors of the estimates would mask any true effect if it did exist.

The few selection experiments from which information is available do support the presence of a positive genetic correlation between measures of growth and reproduction in sheep. In New Zealand, Romney sheep involved in the Ruakura Fertility Flock have been successfully selected for either high or low incidence of multiple births. After a number of generations of selection, ewes of the high line showed a slight advantage in weight at 12 and 14 months of age over ewes of the low line when appropriate corrections were made for maternal effects (Clarke, 1972).

Some evidence is also available for Merinos. In Pattie's (1965b) report of 2-way selection for weaning weight, he suggests that a positive correlation did exist between the selection criterion and multiple births, which counteracted the effects of selection bias for and against twinning in his low and high lines, respectively. Extending the studies of the weaning weight lines reported by Pattie (1965a,b), Barlow and Hodges (1976) were able to show that a positive genetic correlation was evident between weaning weight and reproductive performance of ewe lambs, after selection had been relaxed.

In summary, from the limited evidence available, growth and litter size are apparently positively correlated genetically in sheep, whether litter size is expressed directly as lambs born per ewe lambing, or more indirectly as lambs born per ewe mated. The age of the ewe, which is normally confounded with parity does not appear to make a great deal of difference to the magnitude of the genetic correlation, but the evidence for this is very limited.

1.3. PHYSIOLOGICAL MECHANISMS INVOLVED IN THE CONNECTION OF GROWTH WITH REPRODUCTION IN MAMMALS

1.3.1. General

The discussion so far has concentrated on describing relationships observed in terms of the degree of covariation between traits which can be related to gene action. In this section, the underlying physiological reasons behind the existence of the relationships between traits are examined. The intention is not to comprehensively review all physiological mechanisms that could possibly be involved in mediating relationships between growth and reproduction, but rather to discuss the findings of some of the relevant studies that have been conducted, and to suggest what factors are more likely than most to be involved.

From the earlier sections, the genetic relationship that is consistent across the species examined is that between measures of growth and ovulation rate. From a genetic point of view it is not altogether clear whether favourable or unfavourable relationships exist between fertility (in the narrow sense of the ability to reproduce), reproductive lifespan and measures of growth, partly because of the complicating changes in carcass composition which have occurred in lines selected for body weight or gain. In view of this, physiological discussion in this section is concentrated on understanding the relationships between growth and ovulation rate.

1.3.2. Theories and experimental evidence

It has been suggested that the changes observed in litter size in lines of mice selected for body weight are a result of the relationship being dependent on endocrine links, such as the growth and gonadotrophic hormones of the anterior pituitary gland (MacArthur, 1944). The implication here is that ovulation rate has

been the principal component of litter size that has changed via these endocrine links. As there has been no serious challenge to this suggestion in the last 40 years, it appears to be quite relevant to examine MacArthur's comments further. He suggests that size genes may control primarily the size of the pituitary gland, and hence, secondarily, both body size (by the growth hormone) and litter size (by the follicle-stimulating gonadotrophic principle). However, he also suggests that the size genes are more likely to primarily regulate general body growth, and hence, by laws of relative growth, the size of the pituitary gland, whose gonadotrophic hormone, acting quantitatively, determines the litter size.

These suggestions play down the significance of related changes in ovarian size; MacArthur justifies this on the grounds that the mouse can be easily superovulated with injections of exogenous gonadotrophins or by transplanting pituitary tissue. Hormone action however, involves two main components, which are the hormone's activity or concentration available to act on a target organ, and the organ's sensitivity or responsiveness to it. To determine the contribution made by each of these to the changes in ovulation rate resulting from selection for body weight in mice, Fowler and Edwards (1960) compared females of divergent weight lines for the number of eggs ovulated following injections of exogenous gonadotrophin. The purpose of this method was to find the dose of exogenous hormone required to match ovulation rate following natural oestrus; this dose could then be used to infer the amount of endogenous gonadotrophin present in females of each line. Fowler and Edwards concluded that most of the change in ovulation rate had been due to changes in gonadotrophin activity (i.e. no great change in ovarian sensitivity). This posed the question as to whether alterations in gonadotrophin activity resulted from changes in pituitary gland size, or its potency (or both). Edwards (1962) found that pituitary gland weight was highly correlated to body weight, and that no differences existed in the amount of pituitary

tissue per unit body weight between mice of high and low body weight lines. Furthermore, the unit potency of the pituitary gland was the same in each of the lines. The conflict between Edwards' results and those of Fowler and Edwards (1960) will be dealt with later.

Changes in ovarian sensitivity to gonadotrophins appeared to be the reason for increases in ovulation rate in another line of mice selected for high body weight (Durrant, Eisen and Ulberg, 1980), even though similar methods were used to those of Fowler and Edwards (1960). Lines selected for natural and induced ovulation rate (Land and Falconer, 1969) have been used to show that gonadotrophin activity and ovarian sensitivity are both positively genetically correlated with body weight (Land, 1970).

Complications arise with the above-mentioned studies however, as the exogenous hormone tests used are also influenced by the levels of endogenous gonadotrophins in the intact, test animal (Lamond and Emmens, 1959; Lamond and Bindon, 1966 and Bindon and Pennycuik, 1974) and do not simply measure ovarian sensitivity. To be fair, Bindon and Pennycuik's (1974) results from using a hypophysectomized mouse model (with presumably no source of endogenous gonadotrophin) versus an intact animal for testing ovarian sensitivity did not altogether conflict and were in substantial agreement at the highest dose of gonadotrophin administered.

Given that ovarian sensitivity was probably not accurately reflected in the test used by Fowler and Edwards (1960), perhaps the conflict of their results with that of Edwards (1962) is simply a consequence of wrongly describing changes in ovulation rate wrought by selection for body weight as being due to increased gonadotrophin activity. However, a more plausible explanation would be to also suggest that Edwards' (1962) attempts to measure the potency of the pituitary gland are quite unsatisfactory, in light of more recent knowledge. It is now known that in many

mammals, the gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are released from the pituitary gland either in frequent pulses (LH) or discharges of longer duration (FSH), throughout the oestrous or menstrual cycle, in addition to the pre-ovulatory release of both hormones (see reviews by Reeves, 1980; and Pohl and Knobil, 1982). Both tonic and pre-ovulatory secretion of LH and FSH are a consequence of rhythmic discharges of gonadotrophin releasing hormone (GnRH) from the hypothalamus into the pituitary portal circulation, tonic secretion being controlled by 'negative feedback' effects of oestradiol (Reeves, 1980; Pohl and Knobil, 1982). Progesterone can enhance negative feedback effects of oestradiol on LH (in sheep, Findlay et al., 1978; in monkeys and humans, Pohl and Knobil, 1982) but not that of FSH (in sheep, Scaramuzzi et al., 1971). Feedback effects on the latter hormone could be modified by androgens in the sheep (Martensz, Scaramuzzi and Van Look, 1979); they have been shown to affect FSH release in the rat (e.g. Gay and Tomaccarri, 1974). It has been proposed that tonic FSH secretion in mammals is also subject to feedback effects of inhibin from the ovary (e.g. de Jong, 1979; Welschen, Hermans and de Jong, 1980, in rats); there is increasing evidence for such a pathway in sheep (e.g. Cummins et al., 1983; Tsonis et al., 1983; Wallace and McNeilly, 1985).

Positive feedback effects of oestrogens are responsible for the pre-ovulatory release of both LH and FSH (Reeves, 1980; Pohl and Knobil, 1982). The literature suggests that the tonic secretions of LH and FSH are more influential in determining ovulation rate than the secretion pattern or amount of the pre-ovulatory release of the two hormones (Findlay et al., 1978; and Bindon and Piper, 1984 in sheep).

This brief discussion of the control of gonadotrophin secretion is intended to demonstrate that gross measurements of pituitary gland size, and its potency (such as those used by Edwards, 1962) are unlikely to reflect either the pattern or amount of

gonadotrophin secreted in the normal situation, particularly that relating to tonic levels. As such, gross measurements do not greatly aid attempts at understanding the physiological control of ovulation rate, nor its connection with growth.

Clearly, a more sophisticated approach is required than hitherto used. Present knowledge therefore does not permit definite conclusions to be made about what physiological mechanisms are involved in mediating relationships between ovulation rate and measures of growth.

1.3.3. Potential factors that might be involved in mediating relationships between growth and ovulation rate

1.3.3.1. General

There are seven well-established hormones secreted from the anterior pituitary gland, FSH, LH, growth hormone (GH), prolactin (PRL), adrenocorticotrophic hormone (ACTH), thyroid-stimulating hormone (TSH) and intermedin, and four by the posterior pituitary gland, oxytocin, arginine vasopressin, lysine vasopressin, and arginine vasotocin (Dickson, 1977). The discussion has been limited to 3 hormones (and the hormones they in turn release) for which some information is available, these being prolactin, adrenocorticotrophic hormone and thyroid-stimulating hormone. Growth hormone, LH and FSH have already been mentioned.

1.3.3.2. Prolactin

The potential involvement of prolactin is suggested on the one hand by its apparent involvement with the growth process (e.g. Bates, Milkovic and Garrison, 1964 in rats, Sinha, Lewis and Vanderlaan, 1972a in mice, Ryg and Jacobsen, 1982 in deer, Ohlson, Spicer and Davis, 1981 and Brinklow and Forbes, 1982 in sheep) and on the other by its involvement in ovarian function (see review by

McNeilly, 1984).

Lack of synthesis of prolactin and growth hormone is the primary cause of the dwarf phenotypes of Snell and Ames mice (Slabaugh et al., 1981); females and most males are infertile in the homozygous condition (Bartke, 1967). In species where daylength variation affects growth, prolactin has been shown to be associated, but evidence is lacking as to whether it is causally involved in growth responses (Forbes, 1982 and Brinklow and Forbes, 1984). A correlation between the discharge of prolactin prior to the LH surge and the number of pre-antral follicles has been reported in sheep (Cahill et al., 1981), but whether this is a causal relationship or merely an artefact remains to be clarified. McNeilly (1984) concludes, from an extensive review of information from a number of mammalian species, that there is abundant evidence that prolactin is involved directly at the ovarian level to promote follicular growth and development.

Recent evidence suggests that prolactin is not a single substance, but rather a family of molecular variants. Each form may be a hormone with a different physiologic action, a view that distributes the burden of the perplexing number of functions ascribed to prolactin (Leong, Frawley and Neill, 1983). This view is attractive, but it will only be confirmed or refuted by thorough investigation.

1.3.3.3. Adrenocorticotrophic hormone (ACTH) and adrenal cortex secretions

The principal function of ACTH is to stimulate adrenocortical activity from the adrenal gland, although it is becoming increasingly apparent that it also has roles in fat mobilisation, ketogenesis, muscle glycogen storage, control of blood glucose levels and control of plasma amino acid concentrations (Dickson, 1977). ACTH stimulates the secretion of the hormones cortisol,

corticosterone, cortisone (glucocorticoids), aldersterone (a mineral corticoid) and several others from the adrenal cortex (Dickson, 1977). These hormones are involved with many functions of the body. In all the species where the adrenal cortex has been completely removed by surgery (dog, human, rat, sheep, pig, cow and horse), the results of this operation included weakness, quickness to fatigue, hypotension, marked intolerance to fasting, exaggerated response to insulin, and decreased ability to withstand stress (Dickson, 1977). Direct evidence of the effects that adrenal cortex secretions have in mediating relationships between measures of growth and ovulation rate is lacking. Nevertheless, information is available where the role of these hormones are examined in controlling growth traits and reproductive traits as separate issues.

That the adrenocorticoids might be involved in the control of growth is suggested by anatomical and experimental observations. Corticotrophs and somatotrophs (pituitary cell types) are closely juxtapositioned in pituitary gland tissues (e.g. Denef and Andries, 1983) and experimentally, the administration of dexamethasone (a synthetic glucocorticoid) has enhanced the pituitary gland growth hormone response to GH releasing factor in intact as well as adrenalectomized rats (Wehrenberg, Baird and Ling, 1983). In addition, cultured rat pituitary gland cells respond to thyroid and glucocorticoid hormones by increases in growth hormone production and growth hormone mRNA (Martial et al., 1977). Cortisol, along with prolactin, has been implicated in the growth responses of sheep to differing photoperiods, although, as is the case with the latter hormone, critical evidence of its exact role is lacking (Forbes, 1982; Brinklow and Forbes, 1984).

Turning to reproduction, hyperadrenal states result in depression of ovarian function (see Li and Wagner, 1983a, for references); the results of one study suggest that cortisol has a direct action on the pituitary gland to depress both basal and

stimulated LH release (Li and Wagner, 1983b). However, glucocorticoids could also be acting at the ovarian level, by inhibiting both the expression of LH receptor and the induction of aromatose enzyme in granulosa cells, 2 key steps in the development of pre-ovulatory follicles (Schoonmaker and Erickson, 1983). In another study, factors of adrenal origin were suggested as inhibiting the release of LH (in agreement with Li and Wagner, 1983b) without affecting the release of FSH (Zanisi, Messi and Martini, 1983).

A study where adrenal function and ovulation rate have been examined together has been conducted in sheep. In one experiment, administration of ACTH for three days prior to oestrus increased ovulation rate, in addition to delaying the onset of oestrus and lengthening the oestrus cycle (Doney et al., 1976). In a second experiment, the same protocol of ACTH administration had no significant effect on ovulation rate, whereas continuing the treatment during oestrus reduced it. The administration of either cortisone acetate or Metyrapone (to suppress endogenous cortisol) were without effect on ovulation rate (Doney et al., 1976). These results suggest that adrenocorticoids could play a role in determining ovulation rate, but there is a real need for more critical studies before any definite conclusions can be made.

1.3.3.4. Thyroid-stimulating hormone (TSH) and thyroid gland secretions

While TSH affects all aspects of thyroid hormone synthesis and release, its principal action appears to be colloid endocytosis and hormone release from the thyroid gland (Dickson, 1977).Essentially, the function of the thyroid gland involves the concentration of iodide and the synthesis, storage and secretion of the thyroid hormones. The two thyroid hormones secreted into the blood stream, triiodothyronine (T_3) and thyroxine (T_4) actually include the element iodine in their structure (Dickson, 1977). Thyroid hormones

influence practically every organ in the body, and their involvement in the growth and reproductive functions is widely documented (Dickson, 1977).

As with the discussion on adrenocorticoids, there is no critical information available implicating thyroid hormones in the relationship between measures of growth and ovulation rate, but their roles in growth and reproduction as separate functions have been investigated.

Some of the lines of mice selected for body weight have been studied for changes in thyroid activity. After adjusting for fatness, Edwards (1962) could find no differences in the uptake of I^{131} (a measure of thyroid activity) per unit body weight between the large and small strains originally developed by Falconer (1953, 1955). In contrast, mice from a line previously selected for high body weight in another laboratory had lower values of I^{131} uptake per body weight$^{0.75}$ (metabolic body size) than control mice, which in turn recorded lower values than contemporary mice from a line selected for low body weight (Synenki et al., 1972). The apparent discrepancy in results between the two studies may be due to genetic differences in the lines used or in the assay procedures followed (Synenki et al., 1972). Synenki et al. (1972) concludes that there is a genetic correlation of positive sign between body weight and thyroid activity in mice; it has been pointed out earlier that mice from the same lines differ in ovulation rate among the body weight selections (Elliot et al., 1968).

In lines of mice selected for plasma thyroxine levels, growth responses in young mice appear to have occurred mainly through changes in maternal performance rather than individual growth (Tilakaratne, Hill and Land, 1981). This appears to be at odds with results from 2-way selection in mice based on I^{131}-uptake of the thyroid gland, where after 8 generations, both males and females from the high line had heavier weights post-weaning than mice from

the low line (Chai, 1970). However, it should be noted that selection based on measurements of the thyroid hormones in plasma (used by Tilakaratne et al., 1981) may not necessarily lead to similar correlated responses in body growth as Chai's (1970) selection criterion, due partly to feedback control mechanisms controlling thyroid hormone secretion (see Dickson, 1977) and partly because the traits are to some extent likely to be under separate genetic control.

Further evidence for the involvement of thyroid hormones in growth and reproduction comes from the experimental induction of hypo- or hyper-thyroidism in rats. One problem that arises in making conclusions from such studies is in the danger of using results from abnormal conditions to make inferences about physiological functioning under normal circumstances. However, in the absence of more critical information these studies are still relevant.

Thyroidectomy in rats leads to a rapid and long-lasting decrease in pituitary growth hormone concentration (e.g. Hervas et al., 1975), and a similar result has been obtained by making rats hypothyroid with an iodine-deficient diet containing thiouracil (Wong, Döhler and Mühlen, 1980). In both these reports, injections of T_3 and T_4 stimulated the reaccumulation of growth hormone content in the pituitary gland; Hervas et al. (1975) also report an increase in basal plasma growth hormone levels following T_3 and T_4 administration.

In adult rats thyroidectomy also facilitates LH release (La Rochelle and Freeman, 1974; Dunn, Hess and Johnson, 1976) and leads to a reduction of pituitary LH concentration (Wan and Chen, 1974; Aranda et al., 1976; Suzuki et al., 1978). Both are reversed by treatment with T_3 or T_4 (La Rochelle and Freeman, 1974; Aranda et al., 1976; Suzuki et al., 1978) and Wong, Döhler and Mühlen (1980) report similar findings. Furthermore, it has

been suggested that altered thyroid status acts directly on the hypothalamo-pituitary axis to change the secretion rate of LH, but does not influence the hormone's synthesis or metabolism (Freeman, La Rochelle,Jr. and Moore, 1975).

The work of Bruni et al. (1975) and La Rochelle and Freeman (1974) have both shown that castration of rats influences the effect of hypothyroidism. Thyro-parathyroidectomy superimposed upon castration results in elevated rather than reduced LH (and FSH) levels as in previously intact animals, when the gonadotrophins are measured 30 days after surgery; the levels being higher than that produced by castration alone (Bruni et al., 1975). These levels of gonadotrophins could be restored to those found in the appropriate control animals by administering T_4 (Bruni et al., 1975).

Other reports concerning the effect of hypothyroidism on FSH synthesis and release are not consistent. Whereas La Rochelle and Freeman (1974) and Dun et al. (1976) reported facilitation of FSH release in hypothyroid rats, Baksi (1973) and Suzuki et al. (1978) observed no change, nor could Suzuki et al. (1978) find any change in pituitary gland concentrations of FSH after thyroidectomy. On the other hand, rats made hypothyroid by the feeding of thiouracil had reduced pituitary gland concentrations of FSH, a situation which could be reversed by administration of thyroid hormones (Wong et al., 1980).

The literature therefore does suggest the possibility of a role for the thyroid hormones in mediating relationships between growth and ovulation rate, although critical evidence is lacking for their involvement with the latter.

1.3.3.5. The hypothalamus and the central nervous system

Not only are the adrenal and thyroid glands and their secretions possibilities for investigation; it should be remembered

that the pituitary gland is subject to control from the hypothalamus. Relatively small changes in the frequency of GnRH stimulation (GnRH emanating from the hypothalamus) not only alter the concentration of LH and FSH in the circulation but also have major effects on the ratio of FSH to LH (Pohl and Knobil, 1982). The consequences of these alterations on ovarian function have been described in rhesus monkeys. Pohl and Knobil (1982) in reviewing earlier work state 'A decrease in the frequency of GnRH pulses from the physiologic rate of once per hour to once every 90 minutes appeared to reduce the incidence of ovulatory menstrual cycles. A regimen of one pulse every two hours yielded only anovulatory cycles whereas one pulse every three hours was incapable of inducing follicular development. It should be recalled in this connection that these reductions in GnRH pulse frequencies tend to reduce plasma LH levels while elevating those of FSH.'

Although the exact control of GnRH pulse frequency is unclear, the alterations which occur during the course of selection for growth may well include effects on the secretion of hypothalamic regulating hormones via alterations in pulse frequency. This may ultimately affect ovulation rate by influencing the secretory patterns of LH and FSH from the pituitary gland. In support of this possibility, it is known that transmission of information in and out of the central nervous system appears to be facilitated by the catecholamines. This is of relevance because the catecholamines are important to the secretion of the hypothalamic regulating hormones (Dickson, 1977; Adler et al., 1983).

1.4. CONCLUSIONS

Evidence from numerous mammals suggests a positive relationship between body size and litter size within species. When more critical evidence is examined in mice, pigs and sheep, this relationship is confirmed, although the porcine information is not entirely clear, perhaps due to the emphasis on gilt performance in

many of the studies conducted. Ovulation rate is consistently and positively correlated genetically with measures of growth, more so than litter size itself which is a result of the poor relationship between growth and pre-natal survival.

Reproductive lifespan and fertility may bear negative and therefore unfavourable genetic correlations with measures of growth, but this tentative conclusion is based entirely on studies of lines of mice which had previously undergone long-term selection for body weight, where overfatness may have been a confounding factor. A more thorough investigation of this issue is warranted. Considering the emphasis on carcass traits in selection of pigs, it is of practical interest to know where these traits are favourably or unfavourably correlated genetically with litter size. The literature is unclear on this point, particularly in relation to backfat thickness, but it is suggested that the correlation is probably small in magnitude, if not zero.

The physiological discussion has concentrated on the relationship between growth and ovulation rate. MacArthur's (1944) early suggestions of 'endocrine links' between genes controlling size and those controlling litter size, operating principally from the pituitary gland are still relevant ones. However in more recent times, the field of reproductive physiology has advanced rapidly, and it has become apparent that ovarian sensitivity to gonadotrophins and several additional hormone systems may be involved in mediating relationships between mammalian growth and ovulation rate. The discussion of these have not been exhaustive, but the hormones that could have a role to play, in addition to growth hormone, LH and FSH are prolactin, ACTH (and the hormones secreted by the adrenal gland), and the thyroid hormones. In addition, it is suggested that the central nervous system, and in particular the catecholamines may also have a role to play as they are important in regulating hypothalamic control of the pituitary gland, the pituitary gland ultimately being responsible for

secretion of the gonadotrophins and growth hormone.

Finally, in discussing the similarity of the phenotypic correlation of body weight and litter size in mice with the genetic one, Falconer (1967b) suggests that the relationship may have a simple physiological explanation. This may ultimately be the case, but the path to finding 'the physiological explanation' is likely to be rather complex.

1.5. SUMMARY

Evidence is reviewed from a number of mammalian species on whether a general relationship exists between growth and reproductive performance, firstly by considering phenotypic associations, and secondly by examining more critical information from which inferences can be drawn about genetic relationships. Within each of the following species: rabbits, dogs, dairy cattle, sheep, mice, humans and pigs, the larger the size or higher the body weight of the parent the larger the number of offspring per confinement the parent was reported to have. These positive phenotypic relationships were stronger if ovulation rate was considered rather than the number of offspring.

Within mice, pigs and sheep, there is evidence that the phenotypic associations noted between growth and reproductive performance are a reflection of genetic relationships, particularly of growth with ovulation rate. In mice, the numerous experiments which have been reported where body weight or gain has been the selection criterion were all associated, except one, with correlated changes in first litter size in the same direction as selection. Even in the exceptional case however, ovulation rate had changed in the same direction as selection. Selection for ovulation rate or litter size in mice was, in addition, found to be associated with directional changes in body weight, again in the same direction as selection.

When lifetime reproductive rate has been investigated, mice from lines selected for high body weight have had consistently larger litters, but have stopped reproducing at an earlier age than mice from unselected lines or lines selected for low body weight. This suggests that the genetic connection between growth and litter size (and ovulation rate) is not age-dependent in mice.

The information from pigs is less convincing than that from mice, perhaps as a result of the emphasis on gilts litter size in selection experiments and lack of separation of gilts records from those of older age groups in studies of genetic covariances between relatives. Ovulation rate again appears to be the component of litter size which is positively genetically correlated with measures of growth. There is no clear evidence in pigs of the existence or otherwise of a genetic relationship between backfat thickness and litter size, which suggests that it is probably small in magnitude, if not zero.

In sheep, the evidence suggests that growth and litter size are positively correlated genetically, whether litter size is expressed as lambs born per ewe lambing or as lambs born per ewe mated. The age of the ewe, which is normally confounded with parity does not appear to make a great deal of difference to the magnitude of the genetic correlation.

Given the existence of a general relationship within mammalian species between growth and reproductive performance the review briefly examines what physiological mechanisms might be involved. The suggestion in the early literature of 'endocrine links' between the genes controlling size and those controlling litter size, operating principally from the pituitary gland has been pursued by considering a number of hormones. Early investigators were hampered by the complexity of hormone action, and physiological reasons rather than hypotheses are still not at hand to explain the connection between growth and litter size. However, circumstantial

evidence suggests the possible involvement of prolactin, ACTH (and the hormones secreted by the adrenal gland), the thyroid hormones and the neurotransmitters of the central nervous system in mediating relationships between growth and litter size in mammals. Whatever the connection between the two, growth hormone and the gonadotrophins LH and FSH are likely to play a part because of their respective roles in the determination of growth pattern and ovulation rate.

2. AN INVESTIGATION OF THE CORRELATED RESPONSES IN REPRODUCTION IN LINES OF MICE SELECTED FOR APPETITE, LEAN GROWTH AND FATNESS.

2.1. INTRODUCTION

Reproductive performance is important in determining profitability of many animal production systems, so its genetic determination and inter-relationships with other major traits, namely growth rate, body composition and food intake are important to the animal breeder.

The mouse has been used extensively as a model to help understand the basic genetic and physiological mechanisms involved in traits of importance in larger mammalian species. Reproductive performance has been investigated in outbred populations of mice either by studying lines selected for litter size, or its components, ovulation rate and embryonic survival, or by studying it as a correlated trait to selection for other traits. In almost all published reports of reproductive performance as a correlated trait in mice, selection has been practised for body weight or growth rate (for reviews, see Roberts, 1965, 1979, and McCarthy, 1982). In these published studies, litter size has been used as a measure of reproductive performance, and has usually changed in the direction of selection (e.g. MacArthur, 1949; Falconer, 1953; Rahnefeld, Comstock, Singh and Na Puket, 1966), but not in all cases (Bradford, 1971). Changes in ovulation rate in the same direction as changes in body weight have been shown to be the primary reason for the associated responses in litter size (MacArthur, 1944; Fowler and Edwards, 1960; Land, 1970), although the biological mechanisms involved in these relationships are not understood.

Lines of mice have been selected in the laboratory at the Genetics Department, University of Edinburgh for one of three

criteria, appetite, fat percentage or total lean mass, and are known as the G lines (Sharp, Hill and Robertson, 1984). In this section the correlated responses in litter size after ten generations of selection are reported. To understand these responses in litter size more fully, the major components of litter size, namely ovulation rate and pre-natal survival (including pre-implantation and post-implantation survival) were investigated.

2.2. MATERIALS AND METHODS

(i) Selection lines

Mice were selected for one of three criteria: appetite (A) measured as 4- to 6-week food intake, corrected by phenotypic regression for 4-week body weight, fat percentage (F), using the ratio of gonadal fat pad weight (GFPW) to body weight (BW) in 10-week-old males, and total lean mass (P), using the index BW - (8 x GFPW) in 10-week old males.

For each selection criterion, there were three contemporary lines, one selected for high (H) performance, one for low (L) performance together with an unselected control (C). These lines were replicated three times (replicates 1, 2 and 3) for each of the three selection criteria. Thus, there were 27 lines maintained in all: 3 selection criteria x 3 replicates x 3 directions (H, L and C). Sixteen pair matings were made in each line up to generation 8; subsequently 8 pair matings were used. Selection was practised within litters. In the A lines, both sexes were selected. In the F and P lines, females were taken at random.

A full account of the origins of the mice, selection procedures and the responses obtained in growth, food intake and body composition for the first 11 generations, is given by Sharp et al. (1984). Each generation, 6-week weights, litter size at birth (number of live young) and those born dead were recorded in all the

lines.

Mothers of generations 4 and 10 were given terramycin antibiotic in the water supply for the first week post-partum. This was done to alleviate the effects of an unidentified disease which caused ill-thrift in suckling litters and, in acute cases, death of the mother during the peak of lactation.

(ii) Analysis of ovulation rate and pre-natal survival

Mice and management. Random samples of mice not chosen as parents for the selection lines were taken from each of the 27 lines (replicates 2 and 3 from generation 9 and replicate 1 from generation 10) and pair mated to produce mice for measurement in this study. These mice were thus contempories of those used for breeding in generations 10 (replicates 2 and 3) and 11 (replicate 1) of the selection lines. In addition, a small number of mice not chosen for matings in generation 10 and 11 of the selection lines were used; in the A lines these mice had been measured for the selection criterion. Mothers of generation 10 (Replicate 2 and 3), but not generation 11 (Replicate 1) were given Terramycin antibiotic in their water supply for the first week post-partum. As in the main selection lines, litters were adjusted to between 6 and 12 pups at birth, weaning took place at 21 days of age when the sexes were separated, and weaned mice were held in stock cages (no more than 6 mice in each cage) until mating time.

Females were weighed and mated at 8 weeks of age except in replicate 3 of the F lines which were weighed and mated at 7 weeks, by mistake. Two females were mated to each male, except where close inbreeding could be avoided by pair mating or mating three females to each male. Allocation of mates was similar to a scheme designed by Falconer (1973). The set of three lines, H, L and C of each replicate of each selection criterion were contemporaneous, as during the selection experiment.

Dissection technique. Vaginal plugs were used to indicate the day of mating, and females were dissected after 17 days to measure ovulation rate, pre-natal survival and its components pre- and post-implantation survival. Ovulation rate was estimated by counting the number of corpora lutea on each ovary under a dissection microscope.

This method is liable to underestimate ovulation rate, particularly when the corpora lutea are numerous, because of the difficulty of distinguishing between one large corpus luteum and two adjacent and partially confluent ones. To improve the accuracy of the count, each corpus luteum was dissected out under the binocular microscope with an eye surgeon's scalpel. In 17 out of the 556 pregnant mice studied (3.1%) there were more implants in one horn of the uterus than corpora lutea counted on the adjacent ovary; in these cases the latter count was adjusted upwards to equal the number of implants. Although migration of embryos from one horn of the uterus to the other (McLaren and Michie, 1954) or polyovular follicles (e.g. Kent, 1960) might account for the discrepancy, I considered an error in counting to be a much more likely cause. As this is revealed only where there is no pre-implantation loss in one or both horns of the uterus, a count of corpora lutea could underestimate ovulation rate in more than the 3.1% of cases corrected; but because all lines were counted in the same way, this bias should not seriously affect the conclusions (as argued by Falconer and Roberts, 1960).

The number of live foetuses and post-implantation losses (moles + resorptions + dead foetuses) were also recorded, and percentage survival computed as the ratio of live foetuses to corpora lutea. The number of implants equalled the sum of live foetuses and post-implantation loss sites. Percent pre-implantation survival was computed as the ratio of implants to corpora lutea, and percent post-implantation survival as the ratio of live foetuses to implants.

Statistical analysis. Body weight, ovulation rate, implant number,

live foetus number, pre-natal survival and its components pre- and post-implantation survival were subjected to analyses of variance by least squares. The main model fitted to the dated was:

$$Y_{ijklm} = u + T_i + D_{ij} + R_{ik} + L_{ijk} + F_{ijkl} + e_{ijklm}$$

where Y_{ijklm} is the observation on the mth individual of the lth full-sib family of the kth replicate of the jth direction of selection and the ith selection criterion. Also: u is the overall mean; T_i is the effect of the ith selection criterion (i = 1, 2, 3 corresponding to A, F and P); D_{ij} is the effect of the jth direction of selection (j = 1, 2, 3 corresponding to H, L and C) within the ith selection criterion; R_{ik} is the effect of the kth replicate (k = 1, 2, 3) within the ith selection criterion; L_{ijk} is the effect of the individual line and is used to estimate the effects of drift; F_{ijkl} is the full-sib family effect; and e_{ijklm} is the residual within full-sib family effect.

Directions of selection and replicates were tested against lines, pooled over selection criteria.

In further analyses, terms were also added for linear regression on body weight and/or ovulation rate of the individual mouse.

(iii) Repeat sampling of Replicate 2 of the A lines

The mice used in replicated 2 of the A lines were thought unrepresentative, as indicated by their body weights (see Results). Therefore, using the same procedures an additional study was conducted on this replicate on progeny of mice not selected for generation 12 in each selection line. The mothers of dissected mice did not receive Terramycin antibiotic.

Both the original and the repeat samples contributed to the results analysed, with the repeat sample included as an extra

replicate. The bias created by this procedure was corrected for by reducing the sums of squares for the main effect of replication and the interaction of replication with direction of selection (called 'lines').

2.3. RESULTS

(i) Correlated responses in litter size

The mean litter sizes each generation from Ø to 1Ø are shown in Figures 2.1 - 2.3, for each replicate and for the mean over replicates. To conform with the graphs of Sharp et al. (1984), litter size is plotted against the generation number of the progeny, and represents the reproductive performance of the previous generation of parents.

There was a rapid initial decrease in litter size in all lines. A decrease could be expected between generations -1 and Ø (Ø and 1 of the progeny, Figs. 2.1 - 2.3), as those of generation -1 were a three-way cross (Sharp et al., 1984) with maximum heterosis for litter size. Subsequently, assuming unrelated founder animals, the range in inbreeding coefficients for lines at generation 1Ø was 5.7% to 9.Ø%, with a mean of 6.8% with no consistent difference in inbreeding between selection criteria, directions of selection or replicates. As Falconer's (1973) scheme for minimal inbreeding was used in the selection lines, no inbreeding accrued until generation 4, so inbreeding can not explain the initial decline in reproductive performance. A more likely source of the decline in litter size in the early generations of selection could have been a general decline in the health of the mice, as evidenced by very small young at weaning time and, in acute cases, by deaths of suckling females. Terramycin antibiotic was administered to the mothers of generation 4 and 1Ø, and the offspring of generation 4 had, on average, larger litters than the previous generations.

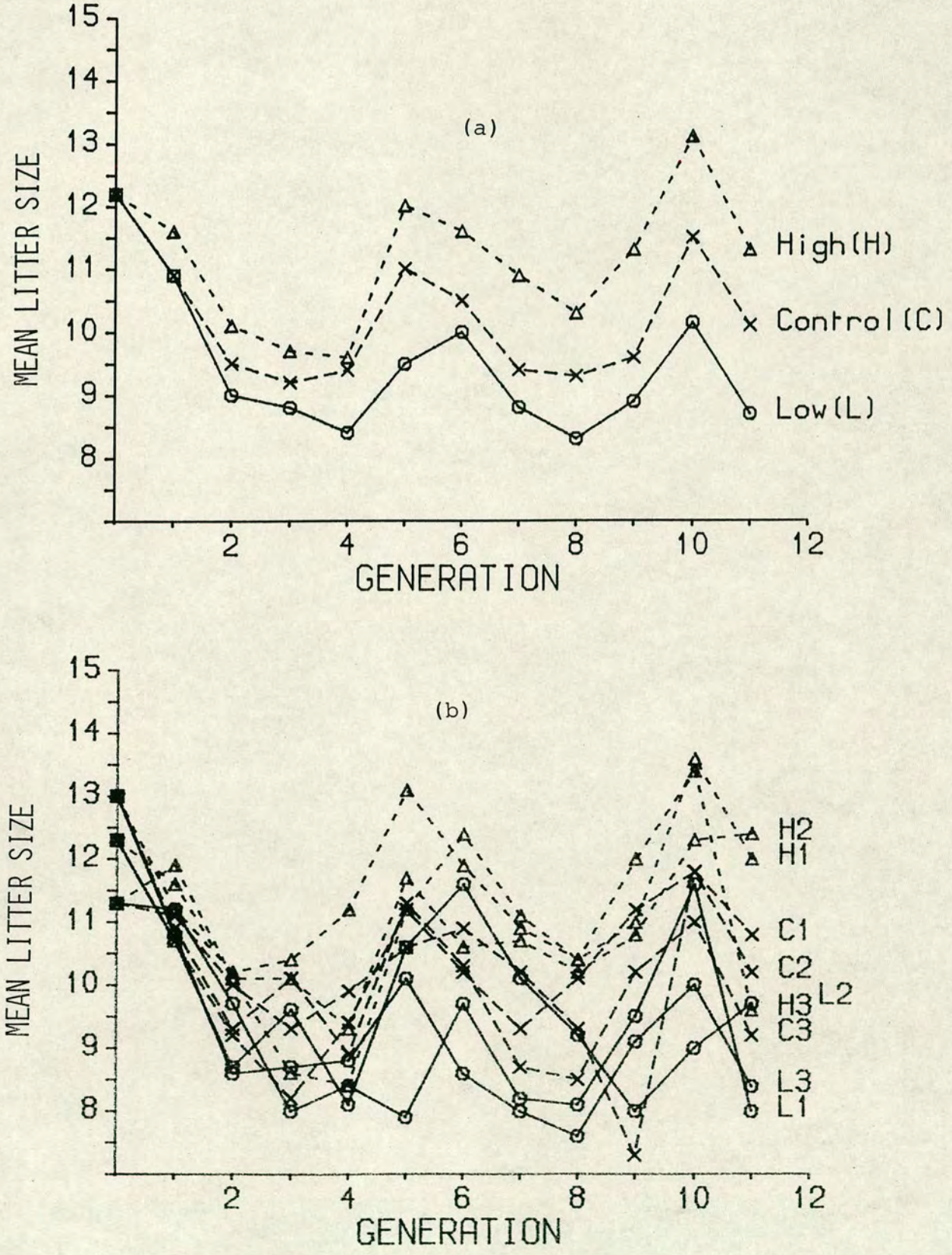


FIGURE 2.1. A (APPETITE) LINES: litter size for (a) mean of all replicates, (b) individual replicates. Generation numbers are those of the progeny to correspond with those of Sharp et al. (1984).

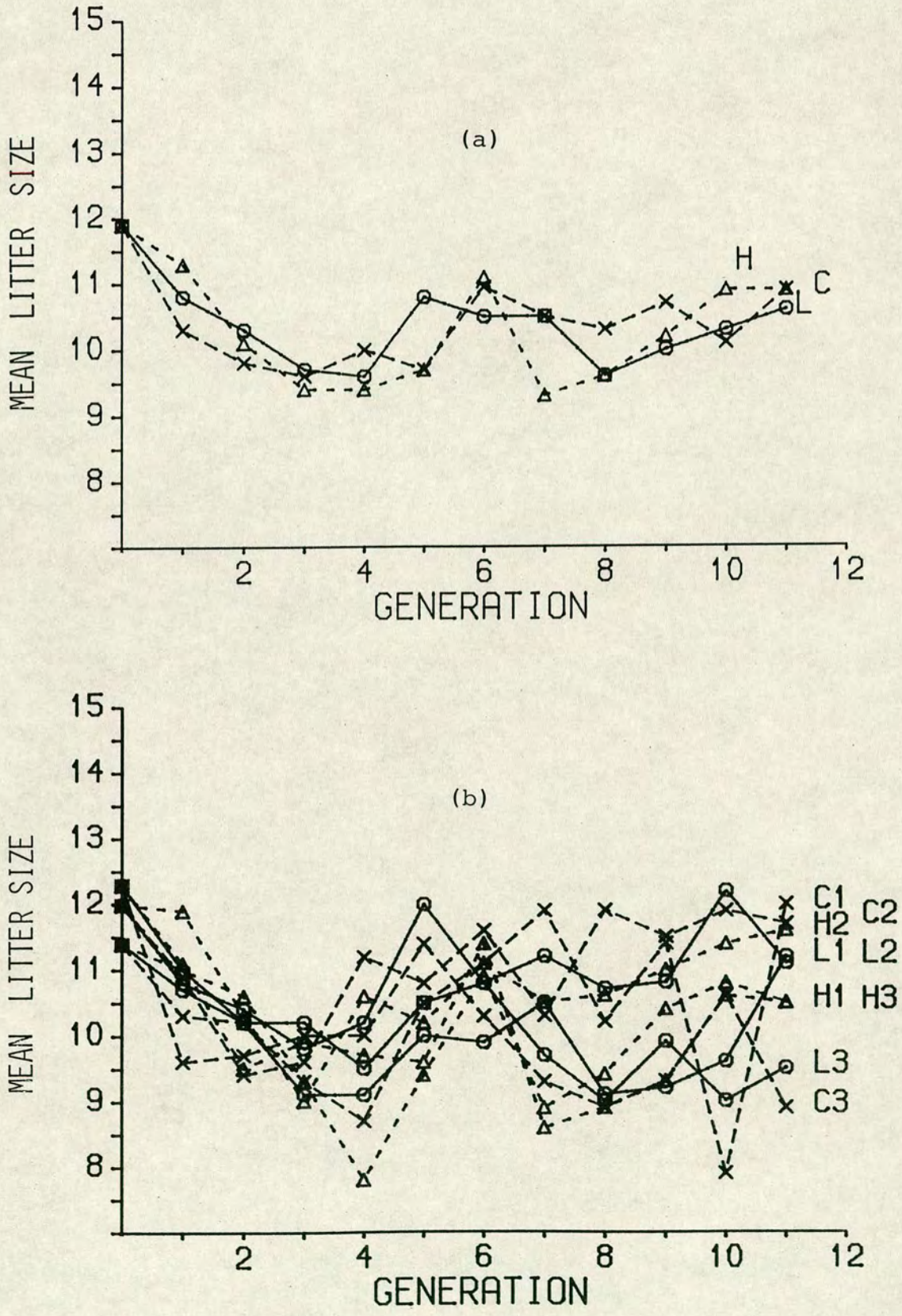


FIGURE 2.2. F (FAT) LINES: litter size for (a) mean of all replicates, (b) individual replicates, as Fig. 2.1.

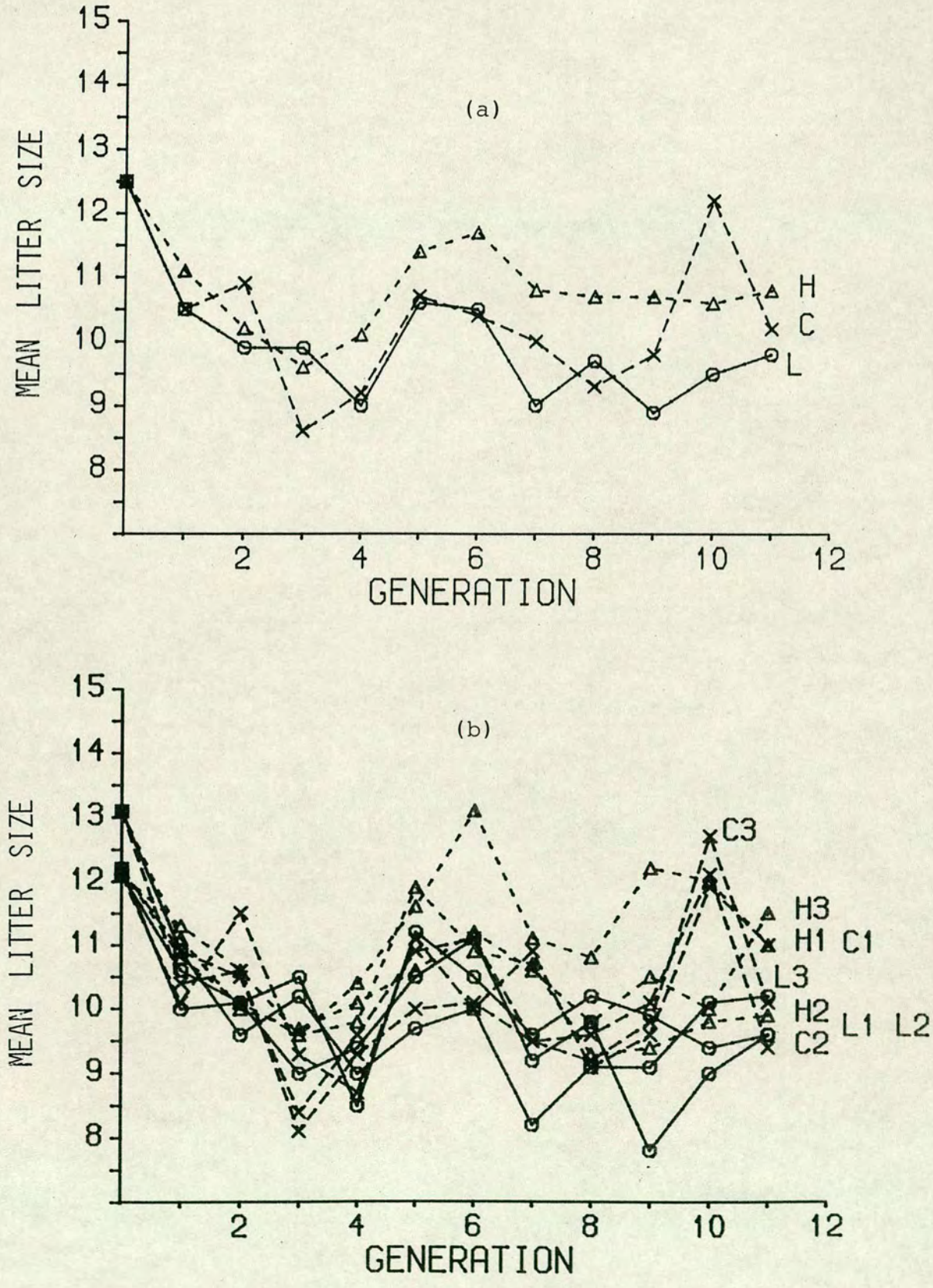


FIGURE 2.3. P (LEAN MASS) LINES: litter size for (a) mean of all replicates, (b) individual replicates, as Fig. 2.1.

Large and consistent differences in litter size between the high, low and control A lines were rapidly established. There were smaller but consistent differences between the high and low P lines, but no consistent differences among the F lines. The difference between the high and low A lines in litter size of generation 1Ø females is 2.6 young born, although the direct character under selection in the A lines has changed relatively less than the selected characters in the P and F lines (see Table 2.1). There is a consistent trend of changes in litter size relative to responses in the selected character appetite, in the A lines (Figure 2.4). The corresponding graph for the P lines is shown in Figure 2.5, but no plot has been given for the F lines where changes in reproductive rate were very small.

At generation 1Ø, the mean difference in 6-week weight of females between high and low selected lines was 3.5g for the A and 6.5g for the P lines (Table 2.1). However, despite these larger differences in female body weights in the P compared to the A lines, the subsequent difference in litter size between the high and low P lines was only 1.Ø young born.

(ii) Ovulation rate and pre-natal survival

Results for ovulation rate and pre-natal survival are given for individual replicates in Table 2.2. Results for the same traits, with the replicates pooled, are given in Table 2.3, together with results for implant number and pre- and post-implantation survival. The analyses of variance are summarized in Tables 2.4 and 2.5 together with the linear contrasts to estimate divergence (H-L) and symmetry ((H+L)/2-C) of response, which were almost orthogonal.

The body weight of females at mating, their ovulation rate, implant number and live foetus number were significantly higher in the high than in the low A lines and although pre-natal survival decreased slightly in the high lines, the difference from the low

TABLE 2.1 Mean of selected character and female 6-week weight in each set of lines at generation 10 (replicates pooled)

Direction of Selection / Selection Criterion	High	Control	Low	High -Low
A (adjusted food intake) (g)*	66.3	63.3	57.5	+ 8.8
6 weeks wt (g)	26.0	23.4	22.5	+ 3.5
F (gonadal fat pad wt/body wt)$^{+}$ (mg/g)	20.5	14.0	8.7	+11.8
6 weeks wt (g)	23.6	23.7	22.2	+ 1.4
P (body wt - 8 x gonadal fat pad wt)$^{+}$ (g)	34.8	29.0	25.6	+ 9.2
6 weeks wt (g)	26.7	22.8	20.2	+ 6.5

* Adjusted food intake (g): FI + 1.65 (16.1 - w) for females,
FI + 2.21 (17.8 - w) for males,
where FI = 4-6 weeks food intake (g)
w = 4 weeks wt (g).

$^{+}$ body weight and gonadal fat pad weights measured in males at 10 weeks of age.

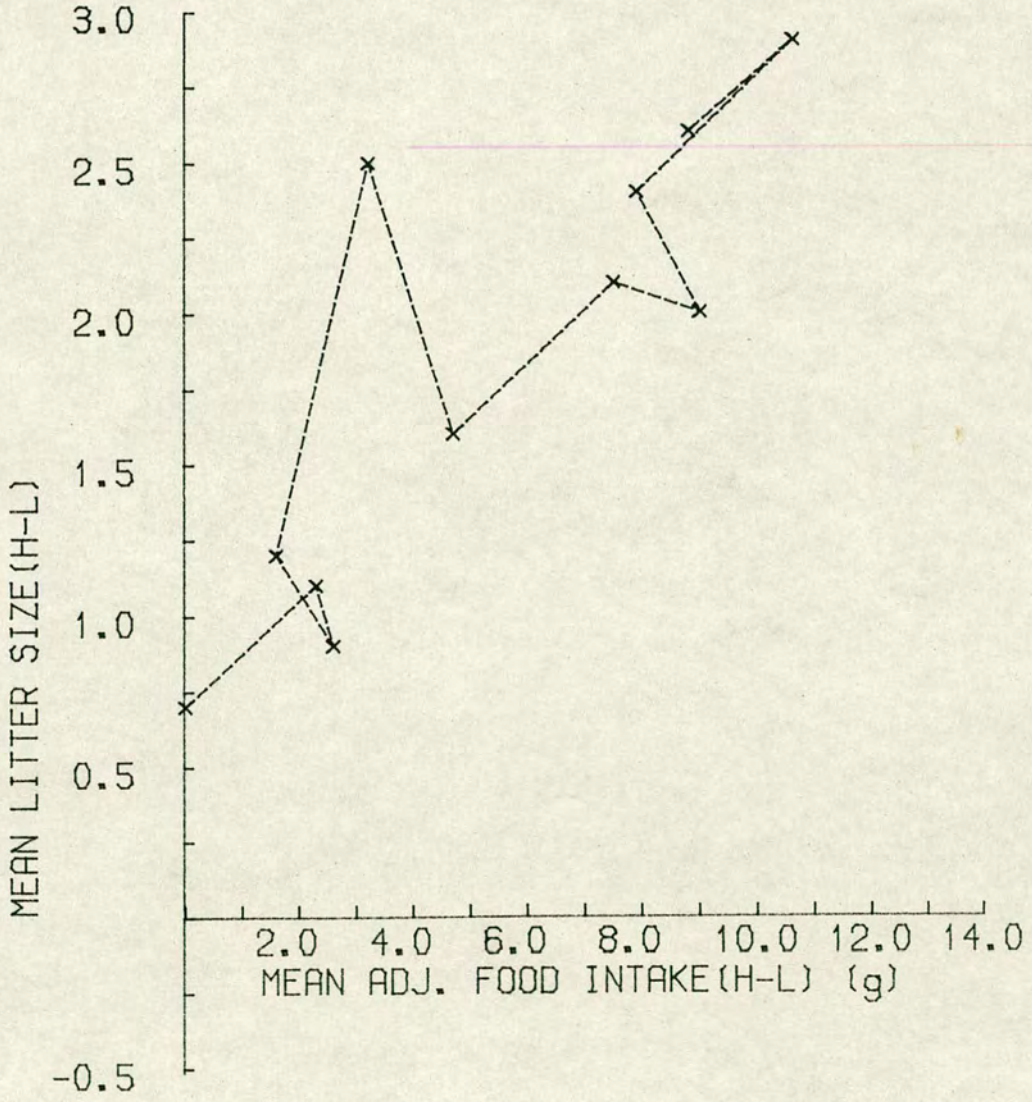


FIGURE 2.4. A LINES: High-low divergence of litter size plotted against high-low divergence of adjusted food intake, for the mean of all replicates.

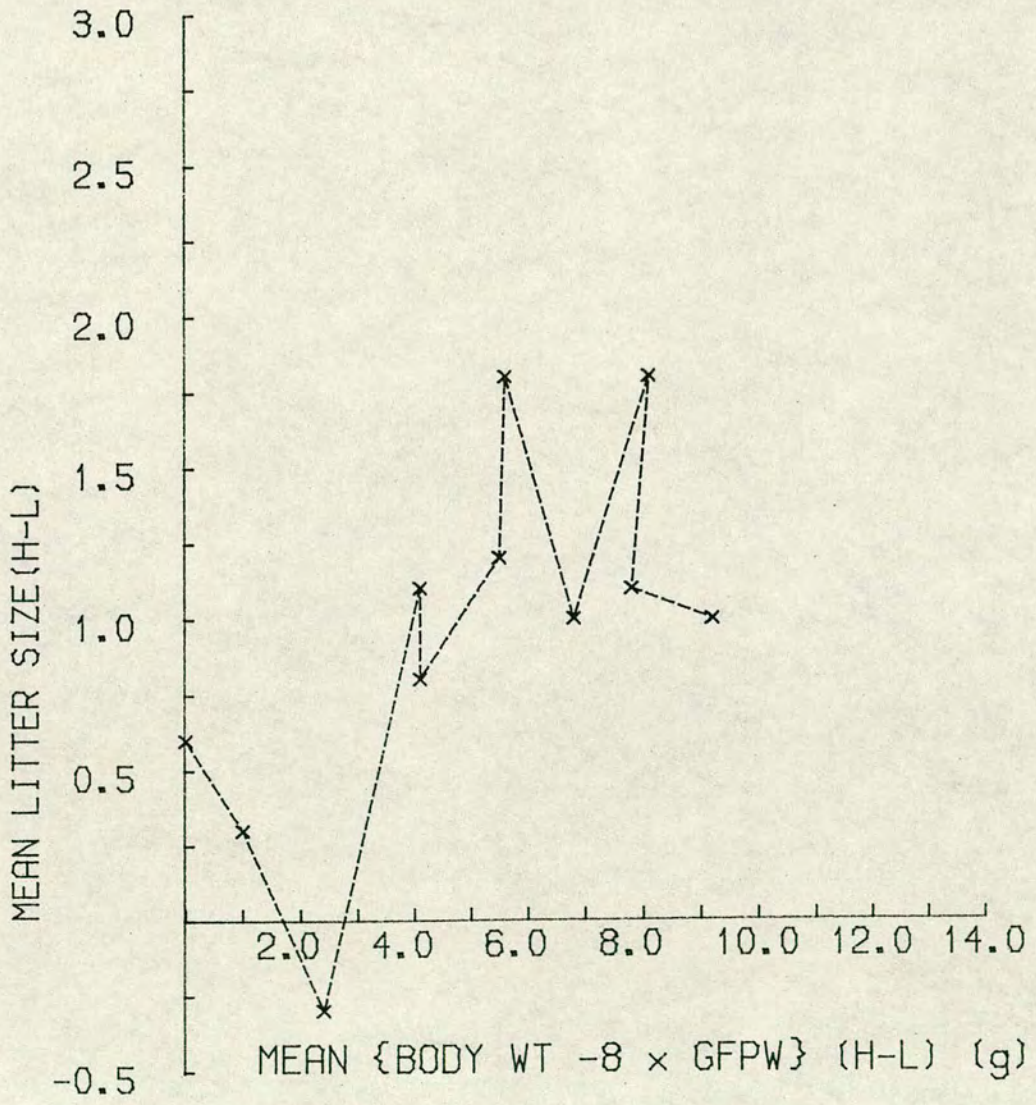


FIGURE 2.5. P LINES: High-low divergence of litter size plotted against high-low divergence of body weight - 8 x gonadal fat pad weight, for the mean of all replicates.

TABLE 2.2 Means for body weight at mating (g) (B.W.), ovulation rate (O.R.), live foetus number (L.F.) and pre-natal survival % (P.S.) (numbers of mice for each mean varied from 14 to 24)

	REPLICATE 1				REPLICATE 2				REPLICATE 3			
Lines	B.W.	O.R.	L.F.	P.S.	B.W.	O.R.	L.F.	P.S.	B.W.	O.R.	L.F.	P.S.
A (adjusted food intake)												
High	28.3	16.9	12.8	80.3	24.6	12.8	11.2	86.8	30.4	17.1	13.4	79.2
Control	24.1	11.9	8.8	73.4	26.1	13.3	11.1	84.0	25.2	11.2	9.4	82.1
Low	24.5	11.9	10.2	86.6	26.8	13.7	11.6	85.4	25.0	12.2	10.1	83.2
A (adjusted food intake)												
High*					30.8	15.8	11.5	73.5				
Control*					24.2	11.9	9.7	81.8				
Low*					24.7	9.6	7.5	78.8				
F (gonadal fat pad wt/body wt)												
High	27.3	12.4	9.6	78.0	27.9	16.2	13.3	83.1	23.4	11.6	10.0	87.5
Control	26.0	13.2	10.0	76.7	27.6	15.5	12.3	79.4	24.2	11.9	10.0	84.8
Low	23.1	14.3	10.3	75.1	24.2	14.2	11.4	80.0	22.1	11.7	9.4	81.9
P (body wt - 8 x gonadal fat pad wt)												
High	29.2	14.8	11.6	78.1	28.3	13.4	11.0	82.5	30.4	15.7	12.6	80.8
Control	20.7	10.8	8.9	82.8	25.0	12.6	10.2	80.8	24.9	12.8	9.9	78.1
Low	21.3	11.3	9.5	84.8	22.3	12.1	10.6	87.5	21.5	11.2	9.2	82.9

*Repeat study

TABLE 2.3 Numbers of mice mated and means of body weight (g) (B.W.), ovulation rate (O.R.), implant number (I.M.), live foetus number (L.F.), prenatal survival % (P.S.), pre-implantation survival (%) (PRE.) and post-implantation survival (%) (POS.) (replicates pooled)

Lines	No.of mice mated	No.of pregnant mice	No.of non-pregnant mice*	MEANS B.W. (g)	O.R.	I.M.	L.F.	P.S. (%)	PRE. (%)	POS. (%)
			A (adjusted food intake)							
High	71	70	1	28.5	15.6	13.1	12.2	79.9	85.4	92.8
Control	74	73	1	24.9	12.0	10.4	9.7	80.3	85.6	94.0
Low	77	77	0	25.2	11.8	10.6	9.8	83.5	89.8	93.2
			F (gonadal fat pad wt/body wt)							
High	62	61	1	26.2	13.4	11.9	11.0	82.9	89.9	91.8
Control	55	51	4	25.9	13.5	11.5	10.8	80.3	85.8	93.7
Low	54	54	0	23.1	13.4	10.9	10.4	79.0	83.1	94.2
			P (body wt - 8 x gonadal fat pad wt)							
High	63	62	1	29.3	14.6	12.7	11.7	80.5	87.1	92.8
Control	59	54	5	23.5	12.1	10.2	9.7	80.6	84.6	94.9
Low	55	54	1	21.7	11.5	10.4	9.8	85.1	90.8	93.7
S.E.+				0.70	0.72	0.50	0.53	2.93	2.61	1.68

* Non-pregnant mice are not included in the analyses.

+ Standard errors of a replicate mean, which are based on between-line variance (except for the 3 survival traits, where it was based on the combined variance of between-lines and between-full-sib family effect).

TABLE 2.4 Linear contrasts for differences between high and low selected lines (H-L) and symmetry, ((H+L)/2-C) and analyses of variance for body weight (g) (B.W.), ovulation rate (O.R.), implant number (I.M.) and live foetus number (L.F.), before and after fitting regressions on body weight

		No Regressions Fitted				B.W. Regressions Fitted		
Contrast	d.f.	B.W. (g)	O.R.	I.M.	L.F.	O.R.	I.M.	L.F.
		Contrasts				Contrasts		
A H-L	1	+3.3**	+3.8**	+2.5**	+2.4**	+2.1**	+1.5	+1.6*
A Symmetry	1	+1.4*	+1.1*	+1.0*	+0.9**	+0.4	+0.5	+0.5
F H-L	1	+3.1**	+0.0	+1.0	+0.6	-1.6	0.0	-0.2
F Symmetry	1	-1.3	-0.2	-0.1	-0.1	+0.5	+0.3	+0.2
P H-L	1	+7.6**	+3.1**	+2.3*	+2.0*	-0.8	-0.1	+0.1
P Symmetry	1	+2.0*	+1.0	+1.4	+1.1	0.0	+0.8	+0.6
						Regression coefficients (±SE)		
B.W.Covariate	1	-	-	-	-	0.49**±0.061	0.42**±0.069	+0.41**±0.072
		Mean Squares				Mean Squares		
Replicates	6	77.77*	67.72*	47.83*	39.99	23.54	23.18	23.00
Lines	12	24.11	22.54*	12.15	13.73	14.46**	8.85	10.57
Families	144	13.28**	9.85**	9.91**	9.62**	6.23**	9.25**	9.71**
Individuals - no regressions	382	3.11	5.13	6.21	6.62	-	-	-
- regressions fitted	381	-	-	-	-	4.38	5.68	6.11

* P < 0.05, ** P < 0.01, otherwise P > 0.05

Tests: Contrasts, main effects and (pooled) replicates against (pooled) lines, (pooled) lines against families and families against individuals.

TABLE 2.5 Linear contrasts for differences between high and low selected lines (H-L) and symmetry ((H+L)/2-C) and analyses of variance for pre-natal survival (%) (P.S.), pre-implantation survival (%) (PRE.) and post-implantation survival (%) (POS.) before and after fitting regressions on ovulation rate.

Contrast	d.f.	No Regressions Fitted			O.R. Regressions Fitted	
		P.S.	PRE. (%)	POS. (%)	P.S.	PRE. (%)
A H-L	1	-3.6	-4.4	-0.3	+2.2	+0.5
A Symmetry	1	+1.0	+1.4	-0.7	+2.7	-2.8
F H-L	1	+3.9	+6.8*	-2.5	+3.9	+6.8*
F Symmetry	1	+0.7	+0.8	-0.7	+0.4	+0.6
P H-L	1	-4.6	-3.7	-0.9	+0.1	+0.2
P Symmetry	1	+2.2	+4.4	-1.7	+3.8	+5.7*
					Regression coefficients (±S.E.)	
O.R. Covariate	1	-			-1.29** ±0.375	-1.37** ±0.349
		Mean squares			Mean squares	
Replicates	6	357	318	203	300	297
Lines	12	143	212	121	201	195
Families	144	447**	347*	142**	416**	334*
Individuals						
No regressions	382	284	248	83.9	-	-
Regressions fitted	381	-	-	-	276	239

* $P < 0.05$, ** $P < 0.01$, otherwise $P > 0.05$

Tests: See footnote to Table 2.4.

lines was not significant. A similar situation was observed for the P lines, except that relative to the A lines, the differences between the high and low lines were larger for body weight at mating and smaller for ovulation rate, implant number and live foetus number. The responses in litter size can therefore be explained by changes in ovulation rate rather than pre-natal survival.

Although the body weight of females at mating was significantly heavier in the high than in the low F lines, ovulation rate, implant number and live foetus number were not different. Pre-natal survival was slightly higher in the high lines, but the difference from the low lines was not significant. However, when the components of pre-natal survival were examined, pre- implantation survival was found to be significantly higher in the high compared to the low F lines. Post-implantation survival on the other hand was slightly lower in the high than in the low F lines, but the difference was not significant.

The linear regression of ovulation rate and live foetus number on body weight at mating (which is a phenotypic, within line regression) completely removed the differences between the high and low P lines, but removed only some of the differences between the high and low A lines.

For the F lines, where the high lines were heavier than the lows, fitting body weight at mating as a covariate made the high (fat) lines appear to have a lower reproductive rate than the lows.

Repeat sampling of replicate 2 of the A lines.

Some circumstantial evidence that the original sampling was unrepresentative comes from comparisons between body weights at mating of 8- week old females in the sample originally dissected 24.6g, 26.1g and 26.8g for H, C and L, respectively, and body weights of 6-week old females in the selection experiment, 25.7g, 22.2g and 24.8g

respectively. Likewise, live foetus numbers in the samples were 11.2, 11.1 and 11.6 respectively and 12.4, 11.1 and 9.7 live young in the selection lines. The High A line in replicate 2 is the only one out of the 27 lines where the 8-week weights of the sample were lower than the 6-week weights from the selection experiment. Assuming a phenotypic regression of at least +0.4 eggs per gram increase in body weight at mating (Land, 1970; Table 2.4), it is not surprising that ovulation rate and live foetus numbers were slightly lower in the original sample of the High than the Control or Low line samples of replicate 2.

The results in the repeat sampling of this replicate were quite different from those obtained previously in both body weight and reproductive performance (Table 2.2), and were more comparable to the results of the selection experiment (Fig. 2.1, Table 2.1).

2.4. DISCUSSION

The results show that changes in ovulation rate, rather than pre-natal survival, are responsible for the changes in litter size in the lines selected for appetite and total lean mass. In contrast, mice selected for percentage fat do not display significant changes in litter size or ovulation rate.

The index used as the selection criterion in the total lean mass lines (body weight - 8 x gonadal fat pad weight) has a very high correlation with body weight and the correlated changes in litter size agree in magnitude with those reported in selection studies on body weight or body weight gain (MacArthur, 1949; Falconer, 1953; Fowler and Edwards, 1960; Rahnefeld et al., 1966; McCarthy, 1982), as do the changes in ovulation rate (MacArthur, 1944; Fowler and Edwards, 1960; Land, 1970).

However, the correlated changes in ovulation rates from selection for appetite are larger than can be explained simply as a

consequence of increases in body weight: For every gram increase in body weight at mating there is an increase of 1.15 (corpora lutea) in the A lines but an increase of only Ø.41 eggs in the P lines. The significant asymmetry in body weight, ovulation rate, implant number and live foetus number of the A lines could be real, but may have been partly due to the relatively low performance of the control mice within the sample dissected, compared to control mice used in other generations.

Fowler and Edwards (198Ø) have suggested from indirect evidence that ovulation rate in the mouse may be correlated more with body protein weight rather than total body weight. Sharp et al. (1984) found that mice from the high A line have become leaner than control mice, but these relatively small differences in carcass composition would only be enough to explain a small part of the higher ovulation rates observed. Further, the F lines with substantially changed composition and significant changes in body weight have shown little change in reproductive performance. So, what could be causing the high correlated responses in reproduction within the A lines? The following explanations are offered as possibilities:

(1) A major gene or genes with large effects on ovulation rate could have been present in the base population, as suggested by the early rapid response in litter size (Figs. 2.1 and 2.4). The evidence for this is, however, unconvincing. The variance of litter size within lines did not show a decline after the first few generations as would be expected following fixation of a major gene. In the study of ovulation rate and pre-natal survival, however, a large variance relative to other lines was noticed for ovulation rate in replicate 1 of the high A line, but this was not consistent for litter size over many generations.

(2) The high A line mice may ovulate more eggs in response to the dynamic effect of consuming relatively large amounts of food ("flushing").

(3) Mice are measured for food intake from 4 to 6 weeks of age. This period encompasses the onset and attainment of puberty, a process which may be physiologically associated with the determination of ovulation rate, general metabolism and of appetite. It is possible that selection for high appetite produced mice which reach their 'peak' of reproductive potential earlier in life than lines selected for body weight, or components thereof.

(4) There may be some pleiotropy between genes controlling food intake and metabolic rate and those controlling ovulation rate. There is evidence of differences in metabolic rate between the high and low appetite selections (S. Bishop, unpublished, M. Nielsen, unpublished).

Interestingly, the increases in ovulation rate in the high A lines is reflected in larger litter sizes, and has not lead to a significant decline in pre-natal survival. A decline in pre-natal survival with increasing ovulation rate has been noted in previous studies (e.g. Bowman and Roberts, 1958; Fowler and Edwards, 1960). The results given in this section can be contrasted with the effects of direct selection where, although ovulation rate has been increased, litter size remained unchanged (Land and Falconer, 1969; Bradford, 1969).

The significantly higher pre-implantation survival in the high compared to the low F lines is suggestive of a genetic association between this trait and fatness per se. There are however several drawbacks to making a definite conclusion. Firstly the gonadal fat pad in relation to body weight has been the trait under direct selection rather than total fat percentage. It is possible that the close proximity of the gonadal fat pad to the reproductive organs is associated with a special relationship between the two, although information on this point is unavailable. Secondly, variation in pre-implantation survival could occur through an

alteration in fertilization rate and from variation in paternal effects, but the evidence suggests that their contribution is small (Fowler and Edwards, 1960; Bradford, 1979).

In conclusion, directional selection for appetite and total lean mass in mice has resulted in changes in litter size and ovulation rate in the same direction as selection, those selected for appetite showing the larger responses. Associated changes in body weight can explain the differences in ovulation rate and litter size in the lean mass lines, but can only partly explain the differences in the appetite lines. Lines selected for percentage fat showed no correlated response in litter size or ovulation rate. The reasons for the large responses in ovulation rate within the appetite lines need closer study and are examined in later sections of this thesis.

A version of this section has been published under joint authorship and a reprint of the paper is attached as an appendix at the back of the thesis.

2.5 SUMMARY

Female reproductive performance is reported in mice selected for 10 generations for one of three criteria, either appetite (A) fat percentage (F) or total lean mass (P). For each criterion lines were selected for high (H) or low (L) performance, with contemporary unselected controls (C). In the A and P lines, litter size changed in the direction of the selected criterion, the changes being larger and more rapidly established in the A than in the P lines. At generation 10, the differences in litter size between high and low lines were 2.6 live young born in the A lines, and 1.0 live young born in the P lines. The differences in 6-week weight between the high and low lines were 3.5g in the A lines, and 6.5g in the P lines. Changes in ovulation rate were the primary reason for changes in litter size, the differences between the high and low lines being

3.8 corpora lutea for the A lines, and 3.1 corpora lutea for the P lines. Fitting body weight at mating as a covariate within lines in the analysis of ovulation rate and live foetus number removed the differences between the high and low selected P lines, but not those in the A lines. The H and L lines did not differ in prenatal survival. There were no consistent differences in litter size or ovulation rate in the F lines.

3. AN INVESTIGATION OF THE REPRODUCTIVE PERFORMANCE OVER FOUR PARITIES IN LINES OF MICE SELECTED FOR APPETITE, LEAN GROWTH AND FATNESS.

3.1. INTRODUCTION

In the preceding section of this thesis, the effects of selection for appetite, lean mass and fatness in mice on first litter size and its major components, ovulation rate and pre-natal survival were examined. However, if inferences are to be made about long-term reproductive rate (the total number of young produced during a major part of a females reproductive life) it is desirable to study more than one litter from each female as, firstly, repeatability of litter size in mice is not high (Falconer, 1981; repeatability = Ø.45, based on first and second litters). Secondly, as an example of the effect of selection for a growth trait, selection for body weight in mice has been reported to depress lifetime reproductive rate, primarily through a shortened reproductive lifespan (Roberts, 1961; Nagai, Harris and McAllister, 198Ø; and von Butler et al., 1984). This is in spite of the general finding that mice selected for body weight have higher first litter sizes than unselected mice (see review, Section 1).

For these reasons it is not clear what effect selection for appetite, lean mass and fatness has had either on long-term reproductive rate or lifetime reproductive rate. I have speculated in Section 2 that the differences in litter size and ovulation rate observed at first litter among the high, control and low appetite lines may be due to a change in the age of the peak of reproductive output, the high lines being advanced and the low lines retarded relative to the control lines. A peak in reproductive rate is normally reached by the second or third litter in unselected populations and a decline is noticeable after the fourth or fifth litter (Biggers et al., 1962; Rapp and Hedrich, 1974).

This section describes a study of four consecutive litters of mice from the appetite, lean mass and fat lines. The study was designed to examine whether differences existed between the selection lines in the reproductive output of mice when measured over a major part of reproductive lifespan, rather than all of it, given the limitations of resources available within the mouse house and the desire to obtain production information from that part of reproductive life which was appropriate in the context of animal production. The study also allowed the survival of the mice to be examined to an age of 7 months and the weights of litters at birth, 12 and 21 days of age to be recorded for use as indicators of maternal ability. At fourth parity (parity is litter order), ovulation rate and pre-natal survival were measured to obtain a fuller understanding of reproductive performance of the selected lines at a time when litter size starts to decline in mice.

3.2. MATERIALS AND METHODS

Source of Female Mice. Female mice from generation 13 of the appetite (A) and total lean mass (P) lines and generation 16 of the lines selected for percentage fat (F) (Sharp et al., 1984) were used in the study. The F lines were examined later as firstly, the A lines had been my primary interest, with the P lines being used for comparison purposes and secondly there was not enough space or labour to cope with a study of the F, A and P lines at the one time. Unlike the P and F lines where selection was conducted on males only, in the A lines, both sexes were selected, so unselected females were used from these lines in addition to selected females to reduce the bias from phenotypic selection.

Mating. The procedures for managing the mice used until they weaned their first litter are those described in detail by Sharp et al. (1984), and earlier in this thesis. In the mating periods for the first and all subsequent parities, males were pair-mated with females for 17 days. Females were weighed and re-bred at intervals

of 7 weeks (starting at 8 weeks of age), or when they had weaned a litter, whichever was earlier. In later parities, pair-mating was not always possible due to occasional deaths of males, so in a small number of cases 2 females were mated to each male. In the A lines, the female mice were re-paired to the same male, where possible. In the P lines, it was not possible to mate females for the second time to the same male, as the males used in the first parity had been sacrificed for measuring the selection criterion. In these lines, the males used in the second and later parities were from the same line as their female mates, care being taken to avoid matings between close relatives. In both the A and P lines, if a female failed to produce a litter after two mating periods, she was paired thereafter with a different male, in an attempt to reduce the influence of male infertility on the results.

In the F lines, 'proven' males were used (i.e. had previously sired a litter) in second and subsequent parities; these were taken at random from the control lines of the G strain. This helped to avoid the possibility of using infertile or inactive males; it was thought that males from the lines selected for high fatness could have become sexually inactive as they approached maturity.

Measurement of traits after birth; disease control. When females gave birth, the number of live and dead young and the total litter weight (alive and dead young) were recorded, litter size being adjusted to between 6 and 12; augmentation of litters was practised rarely, and only when spare young were available from other litters. Following their second and third opportunities to carry a pregnancy to term, dams of litters were given Terramycin antibiotic in their water supply for the first week post-partum. This was done to alleviate the effects of an unidentified disease which caused ill-thrift in suckling litters, and, in acute cases, death of the mother during the peak of lactation. Terramycin was not given to mice following their first litter, as they formed part of the selection lines, and it was not policy at the time to do so. Total litter

weights and numbers present were again measured at 12 days and 21 days of age, the latter age being the time of weaning.

During the last half of the A and P line study, which was conducted at a different time than that of the F lines, ringworm (Trichophyton mentagrophytes) was identified in a small number of mice. Before the end of the study six mice were humanely killed that had shown obvious symptoms of the infection. Rather than kill any more mice before the planned date of dissection, mice of replicates 2 and 3 of the A and P lines were treated for the disease by washing with 'Imaverol' (Crown Chemical Company Ltd., Lamberhurst, Kent, England) 3 times, at intervals of 2 days. At the time, the study of replicate 1 of the A and P lines had almost been completed and so these animals were left untreated.

Dissection. In the fourth mating period, vaginal plugs were used to indicate the day of mating, and females were dissected after 16 days to count corpora lutea (for use as a measure of ovulation rate), the number of implants and live foetuses. Percent pre-natal survival was computed as the ratio of live foetuses to corpora lutea. In addition, percent pre-implantation survival was computed as the ratio of implants to corpora lutea, and percent post-implantation survival as the ratio of live foetuses to implants.

A full description of the dissection technique used is given in Section 2. In 15 of the 282 pregnant mice studied (5.3%) there were more implants in one horn of the uterus than corpora lutea counted on the adjacent ovary; in these cases the latter count was adjusted upwards to equal the number of implants, using the same rationale as developed earlier. In a small number of cases, it was difficult to distinguish what was an active corpus luteum, due to the presence of similar but smaller structures of tissue thought to be corpora albicans. These structures did not occur at a higher frequency in any particular line and it was assumed that if any error did occur in counting due to their presence, the error would

not bias the conclusions.

The decision to commence a study of the F lines was taken too late to measure the first mating weights of females in one of the replicates. Estimated weights were calculated for this replicate (replicate 1) from a prediction based on the regression of 8 week weight on 6 week weight, 6 week-weights being routinely measured in the selection lines.

Statistical analysis.

The survival of the breeding female mice during the study, the percentages having at least 2, 3 or 4 litters of those which had experienced four mating periods and the number of litters produced by each mouse present during all four mating periods were subject to analysis of variance by least-squares, using the model described below in Method 1. The numbers of mice with or without litters following each mating period were analysed by Chi-square, comparing high and low selected lines (H-L) and the symmetry ((H+L)/2 - Control (C)) within each selection criteria, firstly within replicates, and then pooled over replicates.

Litter size information was analysed by three methods, all being analyses of variance by least squares. These were conducted to aid with interpretation of the data, as no one type of analysis could examine all aspects. More detailed reasons for using the three methods are given in the result and discussion sections.

Method 1. Body weight of the dam at mating (B.W.), the interval between pairing and birth (P.B.I.), the number of live young born (A.L.S.), the total number of young born alive or dead (T.L.S.), total litter weight of alive and dead young at birth (BIRTH.WT.), total weights of litter at 12 days (12 DY.WT.) and 21 days of age (21 DY.WT.) were analysed treating information from different parities as being independent. All mice present at mating

contributed to the body weight analyses; for the other traits, records of animals with no litter present or assessable (e.g. cannibalism) at the time the various traits were measured were excluded.

The fourth parity data, B.W., the interval between pairing and the sixteenth day of gestation (the nominal date of dissection) - (also P.B.I.), ovulation rate (O.R.), live foetus number (L.F.), the number of implants (I.M.), pre-natal survival (P.S.), pre-implantation (PRE.) and post-implantation survival (POS.) were also analysed similarly, the information being treated as if it were independent of that from earlier parities. The main model fitted to the data was:

$$Y_{ijklm} = u + T_i + D_{ij} + R_{ik} + L_{ijk} + F_{ijkl} + e_{ijklm}$$

where Y_{ijklm} is the observation on the mth individual of the lth full-sib family of the kth replicate of the jth direction of selection and the ith selection criterion. Also u is the overall mean; T_i is the effect of the ith selection criterion (i = 1, 2, 3 corresponding to A, F and P); D_{ij} is the effect of the jth direction of selection (j = 1, 2, 3 corresponding to H, L and C) within the ith selection criterion; R_{ik} is the effect of the kth replicate (k = 1, 2, 3) within the ith selection criterion; L_{ijk} is the effect of the individual line and is used to estimate the effects of drift; F_{ijkl} is the full-sib family effect; and e_{ijklm} is the residual within full-sib family effect. Directions of selection and replicates were tested against lines, pooled over selection criteria. The exceptions to the model were in the analyses of 12 DY.WT. and 21 DY.WT., where a term was added for curvilinear regression on the number of young present at the time the weights were measured.

In further analyses, terms were also added for linear regression on either body weight of the dam at mating (Parities 1, 2, 3 and 4)

or ovulation rate (Parity 4 only) of the individual mouse.

Method 2. In this case, only B.W., P.B.I., A.L.S. and T.L.S. were analysed; the data comprising only those mice which had 4 litters. Before analysing P.B.I., a value of 3 days was added to the fourth parity figure to make it comparable with the earlier parities, i.e. the fourth parity figure after adjustment was thus measuring the interval between pairing and the nineteenth day of gestation or the expected day of birth. Live foetus number (L.F.) from the fourth parity was used as a putative litter size in the analyses of A.L.S. and T.L.S.

The main model used was:

$$Y_{ijkmnop} = u + T_i + D_{ij} + R_{ik} + L_{ijk} + P_m + (TP)_{im} + (DP)_{ijm}$$
$$+ (RP)_{ikm} + (DRP)_{ijkm} + F_{ijkn} + M_{ijkno} + e_{ijkmnop}.$$

where $Y_{ijkmnop}$ is the observation on the pth record of the oth individual of the nth full-sib family in the mth parity of the kth replicate of the jth direction of selection and the ith selection criterion. Also P_m is the effect of the mth parity (m = 1, 2, 3, 4); $(TP)_{im}$ is the interaction of the ith selection criterion with the mth parity; $(DP)_{ijm}$ is the interaction of the jth direction of selection with the mth parity; $(RP)_{ikm}$ is the interaction of the kth replicate with the mth parity; $(DRP)_{ijkm}$ is the interaction of the jth direction of selection with the kth replicate with the mth parity; M_{ijkno} is the individual mouse effect within full-sib family and $e_{ijkmnop}$ is the residual within mouse effect. The meaning of the other terms was the same as in Model 1. Repeatabilities based on four records were estimated for B.W., P.B.I., A.L.S. and T.L.S., by the following method. The sums of squares for F_{ijkn} (the full-sib family effect) and M_{ijkno} (the individual mouse effect within full-sib family) were pooled to obtain a mean square for the effect of mouse within lines, i.e. the

separate effect of full-sib family was ignored. The expectation for the mean square of mouse within lines was $\sigma^2_W + 4\sigma^2_B$, where σ^2_W is the within mouse between record variance (estimated by the residual within mouse mean square) and σ^2_B is the between mouse component of variance. Repeatability equalled $\sigma^2_B / \sigma^2_B + \sigma^2_W$.

To calculate repeatabilities of traits based on records from contiguous parities, the statistical model (Method 2) was re-run on data containing records of the two parities of interest. Confidence intervals for all repeatability estimates were calculated using the method described by Bogyo and Becker (1963).

Method 3. In this case, two total production traits were analysed, the total number of young born alive (ALVTOT) and the total number of young born alive or dead (ALLTOT), calculated by adding together litter sizes from all the litters produced by each dam, live foetus number from the fourth parity being used as a putative litter size. The only ten records excluded from these analyses were six mice which were killed humanely before the end of the study for health reasons, three mice which had eaten one or more of their litters before the number of young could be assessed and one mouse which was accidently mated only three out of a possible four times. The model used to analyse these traits was the one described in Method 1.

Orthogonal linear contrasts for the differences between high and low selected lines (divergence H-L) and the symmetry ((H+L)/2 - C) of response, both within selection criteria, were performed by analysing the least-square means as observations. Similarly, orthogonal linear contrasts among various parities within the analyses outlined in Method 2 were conducted, and the parity by direction of selection interaction was partitioned into a parity x divergence component and a parity x symmetry component.

3.3. RESULTS

Survival of breeding mice

Least-square means for the survival characteristics of the female mice bred in the study are given pooled over replicates in Table 3.1. The appropriate analyses of variance are summarized in Table 3.2 together with the linear contrasts to estimate divergence (H-L) and symmetry ((H+L)/2-C) of response. Except for the control F lines, which had a lower percentage of mice surviving between the third and fourth mating period than either the high or low F lines or the average of the two (asymmetry, $P < 0.05$), there were no other differences among lines in survival characteristics of the mice at any stage of the study.

Fertility of female mice

In Table 3.3, the arithmetic means for the percentage of female mice having litters of those paired with males are given, pooled over replicates. At none of the four periods of opportunity for females to conceive in the study were there any differences among lines in the percentage of females having litters (Chi-square analysis used). Also in Table 3.3 are least-square means, pooled over replicates expressing the percentage of females having at least two, three and four litters of those which had experienced four mating periods, and the number of litters produced per mouse. The analyses of variance for these traits are summarized in Table 3.4 together with the linear contrasts to estimate divergence and symmetry of response. Except for the lower percentage of mice having four litters in the control P lines compared to either the high or low P lines or the average of the two (asymmetry, $P < 0.05$), which could reflect unusually poor performance of the unselected P line stock, there were no important differences among the lines in the number of litters produced per female.

TABLE 3.1 The number of mice at the start of the study, and least-square means for the percent of breeding females surviving to the start of Mating Periods 2 (2), 3 (3) and 4 (4) and between the mating periods (replicates pooled).

Lines	No. at start	Alive at 2 (%)	Alive at 3 (%)	Alive at 4* (%)	Of those alive at 2: Alive at 3 (%)	Of those alive at 2: Alive at 4 (%)	Alive at 4 of those alive at 3 (%)
				A (adjusted food intake)			
High	48 (45)+	97.8	95.7	95.7	97.9	97.9	100.0
Control	48	100.0	100.0	100.0	100.0	100.0	100.0
Low	48 (47)	97.8	95.6	93.3	97.6	95.2	97.4
				F (gonadal fat pad wt/body wt)			
High	47	93.6	91.4	91.4	97.6	97.6	100.0
Control	39	97.8	93.6	87.4	95.8	89.6	93.2
Low	45	93.8	87.5	84.9	92.9	90.3	97.4
				P (body wt - 8 x gonadal fat pad wt)			
High	24 (23)	100.0	95.8	95.8	95.8	95.8	100.0
Control	24	91.7	91.7	87.5	100.0	95.8	95.8
Low	24	100.0	95.8	91.7	95.8	91.7	95.8
S.E.≠		3.33	5.57	5.94	4.19	4.97	2.06

* All mice surviving to the start of the fourth mating period survived until the end of the study, except one from the A control lines which was killed for health reasons a few days before it was due to be dissected.

\+ Figures in brackets are the number of mice on which percent survival is based, the difference between the bracketed and unbracketed figures being the number of mice killed for health reasons before the start of the fourth mating period.

≠ Standard errors of a replicate mean, which are based on between-line variance.

TABLE 3.2 Linear contrasts for differences between high and low selected lines (H-L) and symmetry ((H+L)/2-C) and analyses of variance for the percentage of mice surviving to the start of Mating Periods 2 (2), 3 (3) and 4 (4) and between the mating periods.

Contrast	d.f.	Alive at 2 (%)	Alive at 3 (%)	Alive at 4 (%)	Of those alive at 2: Alive at 3 (%)	Of those alive at 2: Alive at 4 (%)	Alive at 4 of those alive at 3 (%)
			Contrasts				
A H-L	1	0.0	+0.1	+2.3	+0.3	+2.7	+2.6
A Symmetry	1	-2.2	-4.4	-5.5	-2.2	-3.4	-1.3
F H-L	1	-0.2	+3.9	+6.5	+4.8	+7.3	+2.6
F Symmetry	1	-4.1	-4.2	+0.8	-0.6	+4.4	+5.6*
P H-L	1	0.0	0.0	+4.2	0.0	+4.2	+4.2
P Symmetry	1	+8.3	+4.2	+6.3	-4.2	-2.1	+2.1
			Mean Squares				
Replicates	6	282	478	257	155	403	375
Lines	12	334	1116*	1255*	610**	833	132
Families	159-164	298	537	735	274	481*	234**
Individuals	136-151	326	519	629	235	352	123

*P < 0.05, ** P < 0.01, otherwise P > 0.05.

Tests: Contrasts, main effects and (pooled) replicates against (pooled) lines, (pooled) lines against families if they were significant when tested against individuals, otherwise (pooled) lines were tested against families and individuals combined.

TABLE 3.3 Arithmetic means for the percent of mice with litters following Mating Periods 1, 2, 3 and 4 of those animals present; least-square means for the percent of mice having at least 2, 3 and 4 litters and the number of litters per dam for those which had experienced 4 mating periods (replicates pooled).

Lines	With litters after Mating Period 1 (%)	2 (%)	3 (%)	4 (%)	Had at least 2 litters (%)	3 litters (%)	4 litters (%)	No.of litters per dam
				A (adjusted food intake)				
High	97.9*	91.3	87.0	86.0	100.0	88.4	71.9	3.60
Control	97.9	100.0	85.4	83.0	100.0	93.8	74.9	3.69
Low	91.7	93.6	91.3	93.2	97.9	95.1	75.0	3.68
				F (gonadal fat pad wt/body wt)				
High	93.6	100.0	97.7	97.7	100.0	100.0	87.7	3.88
Control	97.4	100.0	97.2	94.3	100.0	100.0	81.1	3.81
Low	97.8	95.2	97.4	92.1	100.0	100.0	87.5	3.88
				P (body wt - 8 x gonadal fat pad wt)				
High	100.0	100.0	86.4	86.4	100.0	100.0	72.6	3.73
Control	91.7	95.5	77.3	81.0	95.2	89.7	57.1	3.42
Low	95.8	91.7	91.3	86.4	91.1	91.1	86.9	3.64
S.E.+	-	-	-	-	1.96	3.07	8.33	0.11

* Chi-squared analyses were performed on the numbers of mice with and without litters, but arithmetic means are presented as percentages for comparison purposes.

+ Standard errors of a replicate mean, which are based on between-line variance.

TABLE 3.4 Linear contrasts for differences between high and low selected lines (H-L) and symmetry((H+L)/2-C) and analyses of variance for the percent of mice having at least 2, 3 and 4 litters and the number of litters per dam for those which had experienced 4 mating periods.

Contrast	d.f.	Had at least 2 litters (%)	Had at least 3 litters (%)	Had at least 4 litters (%)	No.of litters per dam
			Contrasts		
A H-L	1	+2.1	-6.7	-3.1	-0.08
A Symmetry	1	-1.1	-2.0	-1.4	-0.04
F H-L	1	0.0	0.0	+0.2	0.00
F Symmetry	1	0.0	0.0	+6.5	+0.07
P H-L	1	+8.9*	+8.9	-14.3	+0.08
P Symmetry	1	+0.3	+5.9	+22.6*	+0.26
			Mean Squares		
Replicates	6	181	167	1542	0.195
Lines	12	96.8	274	2145	0.373
Families and Individuals	287	123	430	1634	0.353

* P < 0.05, ** P < 0.01, otherwise P > 0.05.

Tests: See footnote to Table 2.

Parities analysed separately (Method 1)

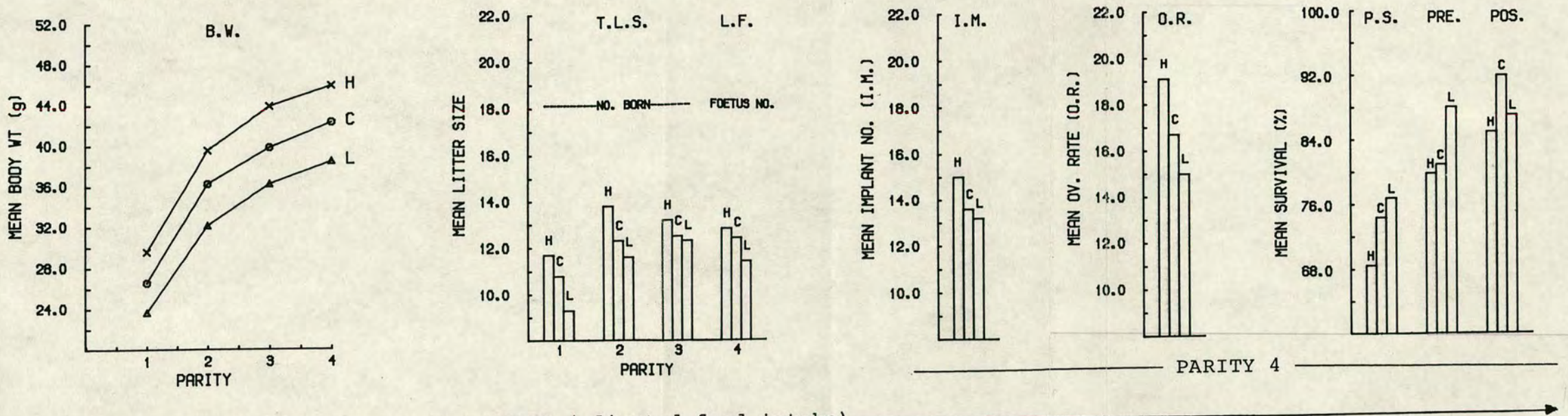
Least-square means, pooled over replicates for body weight and the reproductive traits are shown in Figure 3.1, and those of the other maternal traits are shown in Table 3.5. The trait A.L.S., the number of young born alive is not shown as the values were very similar to T.L.S., the total number of young born alive or dead. No means are shown from the other two analysis methods, as the data used is virtually the same as that used in Method 1. The analyses of variance from Method 1 are summarized in Tables 3.6, 3.7 and 3.8, together with the linear contrasts to estimate divergence and symmetry of response. In the case of Table 3.8, the figures are those after regressions have been fitted on body weight or ovulation rate.

The body weight of dams at each mating and their subsequent litter size following each mating period (A.L.S., T.L.S. and L.F.) were higher in the high compared to the low A and P lines, respectively. In the case of body weight but not litter size, these differences were always statistically significant ($P < 0.01$). Ovulation rates and the number of implants measured after the fourth mating period were also higher in the high compared to the low A and P lines, respectively; the ovulation rate differences being highly significant ($P < 0.01$). In the P lines there was asymmetry ($P < 0.05$) of response for ovulation rate, with the difference between the high and control lines being larger than that between the control and the low lines.

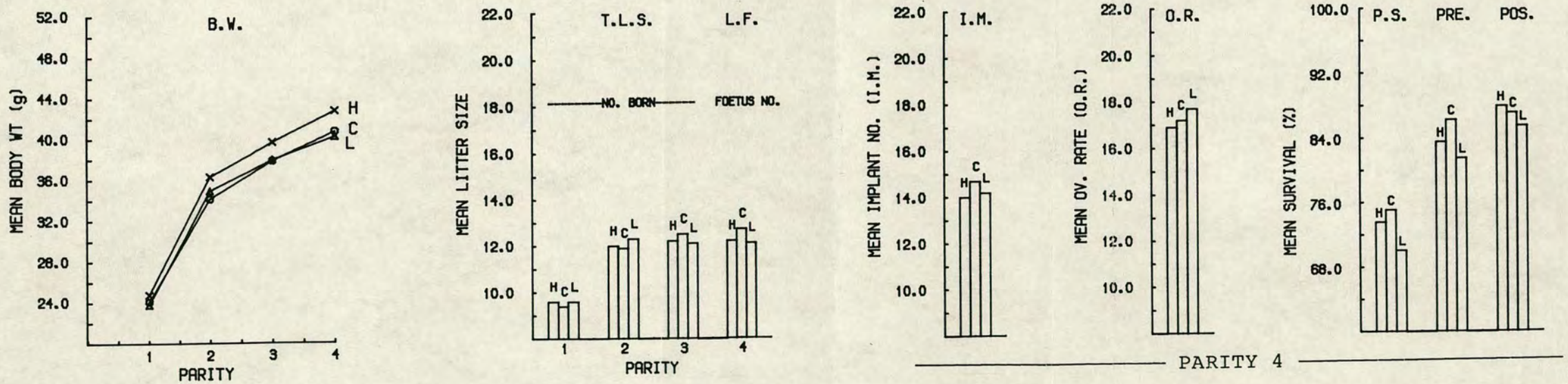
Pre-natal survival was lower in the high compared to the low A and P lines, respectively (significantly so in the P lines, $P < 0.05$), when measured at the end of the study. This could be almost all accounted for by the lower pre-implantation survival observed in the high compared to the low A and P lines, there being only small differences in post-implantation survival.

The linear regressions of A.L.S., T.L.S., O.R., I.M. and L.F. on body weight at the mating period immediately before the measurements

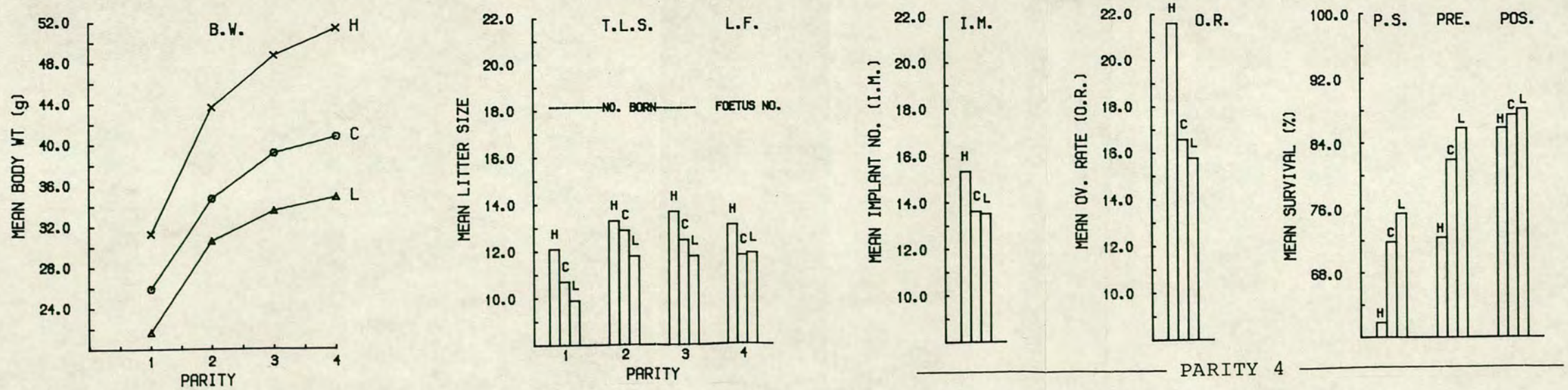
FIGURE 3.1. Least-square means of body weight of dams at mating (g)(B.W.), litter size - either the total number born (alive or dead)(T.L.S.) or live foetus number (L.F.) and for Parity 4 only, implant number (I.M.), ovulation rate (O.R.) and the survival traits, pre-natal survival % (P.S.), pre-implantation survival %(PRE.) and post-implantation survival %(POS.)(replicates pooled).



A LINES (adjusted food intake)



F LINES (Gonadal fat pad wt/body wt)



P LINES (body wt - 8 x gonadal fat pad wt)

TABLE 3.5 Least-square means of the pairing to birth or to day 16 of gestation intervals (P.B.I.), and the weight of litters at birth(g) (BIRTH.WT.),12 days (g) (12 DY.WT.) and 21 days of age (g) (21 DY.WT.) (replicates pooled).

Lines	P.B.I. (days)				BIRTH. WT. (g)			12 DY.WT.≠ (g)			21 DY.WT. (g)		
	P1*	P2	P3	P4	P1	P2	P3	P1	P2	P3	P1	P2	P3
					A (adjusted food intake)								
High	22.3	22.0	22.6	19.0	19.8	23.9	22.4	64.1	72.4	75.7	97.3	115.7	122.9
Control	22.8	21.7	23.5	21.0	16.6	19.4	19.7	62.1	69.9	69.8	95.1	111.3	111.1
Low	22.2	21.1	21.8	20.0	14.5	18.1	18.9	57.7	63.8	66.5	90.9	105.1	105.8
					F (gonadal fat pad wt/body wt)								
High	22.9	21.4	22.4	18.7	14.7	18.5	18.5	59.1	66.5	61.3	91.6	106.0	95.1
Control	22.1	21.2	22.2	21.3	15.1	18.2	18.8	59.2	64.8	61.0	86.5	101.6	94.4
Low	22.3	21.3	21.3	18.8	15.5	19.1	18.9	59.5	64.6	61.2	88.5	99.4	91.8
					P (body wt - 8 x gonadal fat pad wt)								
High	23.6	22.7	22.6	19.7	22.1	24.1	24.1	67.8	75.5	79.2	95.7	120.0	128.7
Control	23.7	22.7	24.4	22.7	17.1	19.8	18.5	56.5	65.5	64.8	80.8	99.4	95.3
Low	22.8	21.2	21.3	18.7	14.9	16.7	16.9	54.9	59.1	61.4	83.8	94.3	98.0
S.E.+	0.67	0.65	1.27	1.09	0.90	1.01	1.01	2.43	2.59	2.73	5.54	6.58	6.92

* P1 = Parity 1, P2 = Parity 2, P3 = Parity 3, P4 = Parity 4.

+ Standard errors of a replicate mean, which are based on between-line variance

≠ Both 12 DY.WT. and 21 DY.WT. least-square means are those from analyses where curvilinear regressions on litter size at the time of measurement have been fitted.

TABLE 3.6 Linear contrasts for differences between high and low selected lines (H-L) and symmetry ((H+L)/2-C) and analyses of variance for the traits in Figure 3.1, and for the number of young born alive (A.L.S.) in Parity 1, 2 and 3.

		B.W. (g)				A.L.S.			T.L.S.								
Contrast	d.f.	P1+	P2	P3	P4	P1	P2	P3	P1	P2	P3	L.F.	O.R.	I.M.	P.S. (%)	PRE. (%)	POS. (%)
A H-L	1	+6.0**	+7.4**	+7.6**	+7.4**	+2.1*	+2.3*	+0.5	+2.3*	+2.2*	+ 0.9	+1.4	+4.1**	+1.8	-8.3	-8.2	-2.1
A Symmetry	1	+0.1	-0.4	+0.2	-0.2	-0.3	+0.5	+0.4	-0.3	+0.4	+0.2	-0.3	+0.4	+0.5	-1.7	+2.9	-6.0
F H-L	1	+1.0	+1.4	+1.7	+2.5	+0.3	0.0	+0.4	0.0	-0.3	+0.1	+0.1	-0.8	-0.3	+3.5	+2.0	+2.5
F Symmetry	1	+0.2	+1.5	+1.0	+0.7	+0.1	+0.2	-0.6	+0.2	+0.2	-0.4	-0.6	+0.2	-0.6	-3.3	-3.7	-0.4
P H-L	1	+9.6**	+13.0**	+15.2**	+16.5**	+2.1*	+1.8	+1.7	+2.2*	+1.5	+1.9*	+1.2	+5.8**	+1.8	-13.5*	-13.4*	-2.4
P Symmetry	1	+0.7	+2.5	+2.0	+2.4	+0.7	-0.4	+0.8	+0.3	-0.3	+0.2	+0.6	+2.1*	+0.8	-3.2	-2.8	-0.4
S.E.≠		0.84	1.19	1.42	1.72	0.57	0.59	0.58	0.54	0.59	0.53	0.73	0.71	0.78	4.05	3.41	2.61
								Mean Squares									
Replicates	6	29.6	83.0	49.3	119	8.7	5.1	37.5*	10.1	4.5	15.1	11.7	23.7	8.9	552	367	137
Lines	12	30.00**	55.5**	85.6**	107**	10.5	9.2	10.9	9.9	9.5	8.9	14.4	13.1	15.8	504	347	166
Families	145-165	6.5**	11.6**	15.8**	19.5**	6.2	7.5	8.5	6.3	7.1	6.7	9.0	7.6*	9.4	317	294	122
Individuals	110-155	2.6	7.2	8.5	10.9	5.7	7.5	6.9	5.0	6.4	6.1	10.1	5.1	11.1	346	315	128

* $P < 0.05$, ** $P < 0.01$, otherwise $P > 0.05$.

Tests: See footnote to Table 2.

+P1 = Parity 1, P2 = Parity, P3 = Parity 3, P4 = Parity 4

≠ Standard errors of a replicate mean, which are based on between-line variance.

TABLE 3.7 Linear contrasts for differences between high and low selected lines (H-L) and symmetry ((H+L)/2-C) and analyses of variance for the traits in Table 3.5.

		P.B.I. (days)				BIRTH.WT. (g)			12 DY.WT. (g)			21 DY.WT. (g)		
Contrasts	d.f.	P1[+]	P2	P3	P4	P1	P2	P3	P1	P2	P3	P1	P2	P3
A H-L	1	0.0	+0.9	+0.8	-1.0	+5.3**	+5.9**	+3.5*	+6.4	+8.6*	+9.2*	+6.4	+10.6	+17.1
A Symmetry	1	-0.5	-0.1	-1.3	-1.5	+0.6	+1.6	+1.0	-1.2	-1.8	+1.4	-1.0	-0.9	+3.3
F H-L	1	+0.6	+0.1	+1.1	-0.1	-0.8	-0.6	-0.5	-0.4	+1.8	+0.2	+3.1	+6.6	+3.4
F Symmetry	1	+0.5	+0.2	-0.3	-2.6	0.0	+0.6	-0.1	0.0	+0.8	+0.3	+3.6	+1.1	-0.9
P H-L	1	+0.8	+1.5	+1.3	+1.0	+7.1**	+7.4**	+7.2**	+12.9**	+16.4**	+17.8**	+11.9	+25.7*	+30.7**
P Symmetry	1	-0.5	-0.8	-2.5	-3.5*	+1.4	+0.6	+2.0	+4.9	+1.8	+5.5	+9.0	+7.8	+18.0
										Regression coefficients (±S.E.)				
Covariates	1													
-Linear (number in litter)									+2.34** (±0.292)	+3.56** (±0.296)	+3.83** (±0.368)	+3.46** (±0.648)	+4.79** (±0.548)	+5.90** (±0.605)
-Quadratic (number in litter)	1								-0.36** (±0.059)	-0.32** (±0.061)	-0.28** (±0.086)	-0.50** (±0.143)	-0.73** (±0.172)	-0.77** (±0.165)
								Mean Squares						
Replicates	6	15.6**	7.5	14.8	28.3	26.7	28.7	70.2	189	351	393	477	2416	2770
Lines	12	1.2	12.4	34.5**	26.4*	29.6*	30.6*	29.5*	211**	225**	233**	1074**	1401**	1390**
Families	146-163	10.1*	7.6	11.9	15.5	15.0*	16.6	15.8	56.2	74.6**	96.8**	323	269	300**
Individuals	106-143	7.0	7.2	9.6	12.4	10.6	13.3	14.6	47.2	39.3	38.0	333	217	171

*P < 0.05, ** P < 0.01, otherwise P > 0.05.

Tests: See footnote to Table 2.

[+] P1 = Parity 1, P2 = Parity 2, P3 = Parity 3, P4 = Parity 4.

TABLE 3.8 Linear contrasts for differences between high and low selected lines (H-L) and symmetry ((H+L)/2-C) and analyses of variance for the traits in Figure 3.1 and Table 3.6 (except for post-implantation survival) after fitting regressions on body weight of dams at mating or on ovulation rate.

		B.W. regression fitted									O.R. regression fitted	
			A.L.S.			T.L.S.		L.F.	O.R.	I.M.	P.S.	PRE.
Contrast	d.f.	P1[+]	P2	P3	P1	P2	P3	P4			(%)	(%)
A H-L	1	0.0	+1.2	+0.7	-0.3	+0.9	+0.7	+0.6	+1.8*	+0.7	+2.2	-0.1
A Symmetry	1	-0.3	+0.5	+0.4	-0.4	+0.4	+0.2	-0.3	+0.3	+0.5	-0.8	+3.7
F H-L	1	0.0	-0.2	+0.4	-0.4	-0.6	0.0	-0.1	-1.5	-0.6	+1.5	+0.5
F Symmetry	1	0.0	0.0	-0.5	0.0	-0.1	-0.4	-0.7	-0.1	-0.7	-2.9	-3.4
P H-L	1	-1.1	-0.2	+2.0	-1.8	-0.8	+1.6	-0.2	+1.3	-0.4	+1.5	-1.9
P Symmetry	1	+0.4	-0.8	+0.9	0.0	-0.8	+0.2	+0.4	+1.2	+0.4	+2.1	+1.3
Covariate		Regression coefficients (±S.E.)										
B.W.	1	+0.34** (±0.062)	+0.15** (±0.053)	-0.02 (±0.049)	+0.43** (±0.058)	+0.18** (±0.050)	+0.02 (±0.044)	+0.09 (±0.049)	+0.29** (±0.037)	+0.14** (±0.050)	- -2.58* (±0.415)	- -1.99** (±0.409)
O.R.	1	-	-	-	-	-	-	-	-	-		
		Mean Squares										
Replicates	6	3.5	6.6	37.7*	5.2	8.8	14.2	9.3	12.2	6.0	359	226
Lines	12	8.2	8.3	11.0	7.3	7.8	8.6	15.7	9.1	15.3	432	345
Families 145-162		5.5	7.4	8.6	5.3	6.8	6.9	8.8	5.8	8.9	255	306
Individuals 109-142		5.4	7.3	6.7	4.4	6.2	5.8	10.1	4.5	11.2	330	315

* $P < 0.05$, ** $P < 0.01$, otherwise $P > 0.05$.

Tests: See footnote to Table 2.

[+] P1 = Parity 1, P2 = Parity 2, P3 = Parity 3, P4 = Parity 4.

were taken (they were phenotypic, within line regressions) removed most of the differences between the high and low P lines, but removed, in general, only some of the differences between the high and low A lines. It should be noted that the regressions of A.L.S. and T.L.S. on body weight of dams in the third parity are not significant; the fit of the model being poor (Table 3.8). Using ovulation rate as a covariate removed all the differences in pre-natal and pre-implantation survival between the high and low A and P lines respectively.

In contrast, although body weights at each mating in females of the high F lines were higher than those of the low F lines, the differences were never significant ($P > 0.05$). In addition, no consistent differences could be detected between the high and low F lines for A.L.S., T.L.S. and L.F. Furthermore, when the mice were dissected, no differences between the high and low F lines could be detected in O.R., I.M., P.S., PRE. and POS..

Weights of litters at birth, 12 and 21 days of age were higher in the high compared to the low A and P lines, respectively. Weight of the litter at birth is highly correlated with litter size at birth, so the differences observed are very much what would be expected given the changes that have occurred in litter size among the A and P lines. It should be noted that 12 and 21 day weights of litter have been adjusted by curvilinear regression for the number of young present at measurement, and that Terramycin administration following the birth of second and third litters may have been responsible in part for the larger differences among lines for litter weights at that time than differences recorded following the birth of first litters.

There were no significant differences between the high and low fat lines for the weights of litters at birth, 12 and 21 days of age, although the high fat line did have a small consistent advantage in 21 day weight of litter. There were no consistent or

large differences between the high and low A and F lines, respectively in the time taken between pairing females with males and birth (time taken between pairing and day 16 of gestation in the final parity studied), although the high P lines took longer to produce litters (or to reach the sixteenth day of gestation) than the low P lines ($P > 0.05$). In both the A and P lines, the control lines took longer than the average of their contemporary high and low lines to produce a litter, but this difference was only significant in the P lines in the last parity studied (asymmetry $P < 0.05$).

Parities analysed together (Method 2)

Analyses of variance from Method 2, together with linear contrasts comparing the 4 parities, in addition to the contrasts already mentioned for Method 1 are shown in Table 3.9.

The most important results from Method 2 analyses are those which come from examining aspects of the data which could not be addressed directly by Method 1. In particular, one aspect of this nature is whether parity influences the differences between high and low selected lines and whether it influences the symmetry of response. These are measured formerly by partitioning the parity x direction of selection interactions computed when using the Method 2 model. Regardless of selection criteria, the parity by divergence and the parity by symmetry interactions were small and non-significant for the two measures of litter size used and for the intervals between pairing and birth.

There was evidence in the P lines that the body weights of dams at mating became progressively greater from Parity 1 to 4 than weights of dams in the low lines (parity x H-L interaction, $P < 0.01$; high versus low line differences of body weights at Parity 3 and 4 were 4.0 g larger than those at Parity 1 and 2, $P < 0.01$).

TABLE 3.9 Linear contrasts for differences between high and low selected lines (H-L), symmetry ((H+L)/2-C), Parity 1 versus 2 (P1-P2), Parity 3 versus 4 (P3-P4), Parities 1 and 2 versus Parities 3 and 4 ((P1+P2)/2-(P3+P4)/2) and analyses of variance for the body weight of dams at mating (g) (B.W.), the pairing to birth interval (days) (P.B.I.), the number of young born alive (A.L.S.) and the total number of young born alive or dead (T.L.S.) from analysis Method 2.

Contrast	d.f.	B.W. (g)	P.B.I.+ (days)	A.L.S.	T.L.S.
A H-L	1	+6.7**	0.0	+1.4	+1.5
A Symmetry	1	0.0	-0.8*	+0.3	+0.1
F H-L	1	+1.8	+0.4	+0.2	-0.1
F Symmetry	1	+0.5	-0.4	-0.2	-0.1
P H-L	1	+13.2**	+1.2**	+1.9*	+1.9*
P Symmetry	1	+1.9	-1.3**	+0.6	+0.2
P1 - P2	1	-10.2**	+1.1*	-2.3**	-2.4**
P3 - P4	1	-2.3**	-1.0*	-0.3	+0.2
P1 + P2 - P3 - P4 / 2	1	-10.1**	-0.6	-1.1**	-1.1**
		Mean Squares			
A PARITY x (H-L)	3	5.0	7.4	13.6	11.0
A PARITY x Symmetry	3	0.65	6.2	7.6	5.4
F PARITY x (H-L)	3	7.2	3.2	0.06	0.22
F PARITY x Symmetry	3	3.0	20.1	3.1	3.4
P PARITY x (H-L)	3	61.4**	0.43	2.3	2.0
P PARITY x Symmetry	3	5.6	13.5	4.3	1.8
Replicates	6	240	9.1	33.7	21.6
Lines	12	207**	7.1	19.8*	20.5**
Parity x Lines	36	9.5**	17.7**	7.8	7.4
Families	131	37.0**	13.1*	14.8	13.6
Mice within families	88	17.0**	8.5	13.3**	12.2**
Individual records	681	3.7	8.1	6.0	5.3

*P < 0.05, ** P < 0.01, otherwise P > 0.05.

Tests: Contrasts of H-L and symmetry and main effects of direction of selection and replication against (pooled) lines, contrasts of parities and parity by direction of selection interactions, parity main effect and (pooled) lines against (pooled) parity by line interaction, (pooled) parity by line interaction against individual records, families against mice within families and mice within families against individual records. If either families or mice within families were not significant effects, they were combined and tested against individual records.

+ The fourth parity figure was adjusted upwards by 3 days to make it comparable with earlier parities.

In general terms, there is good agreement between the results of analyses using Method 1 and 2. The litter size differences between the high and low P lines are relatively larger and the differences between the high and low A lines relatively smaller using Method 2 compared to using Method 1, whereas use of either method did not change the magnitude of the differences in litter size in the F lines. The records of mice without four litters were excluded in Method 2 analyses, and as there were a higher proportion of such records in the study of the A and P lines than in the study of the F lines (see Table 3.3), there would have been a less opportunity of bias from excluding records to influence the results in the latter.

In addition to confirming the longer time it took for the control A and P lines to produce litters than the average of their contemporary high and low lines (asymmetry, $P < 0.5$ for the A lines, $P < 0.01$ for the P lines) the results from Method 2 analyses also indicate that the high P lines took longer to produce litters than the low P lines ($P < 0.01$).

Analysis of litter size summed over parities (Method 3)

Analyses of variance from Method 3, together with linear contrasts comparing divergence and symmetry of response are presented in Table 3.10. By including records of animals which died before the end of the study or suffered infertility, this type of analysis attempts to draw together into the one trait most of the components of the animal which are important in being able to reproduce. Despite their differences in emphasis the results from Method 3 do not conflict in general with those from Method 1 or 2, although there have been slight alterations.

When litter size is summed over parities, the high F lines have a higher value than the low F lines, but the difference was not significant ($P > 0.05$). The presence of a higher percentage of

TABLE 3.10 Linear contrasts for differences between high and low selected lines (H-L), symmetry ((H+L)/2-C) and analyses of variance for the sum over parities of young born per female for young born alive (ALVTOT) and for young born alive or dead (ALLTOT).

Contrast	d.f.	ALVTOT	ALLTOT
		Contrasts	
A H-L	1	+4.6	+5.0*
A Symmetry	1	-1.3	-1.5
F H-L	1	+2.8	+2.0
F Symmetry	1	-0.9	-0.7
P H-L	1	+7.2*	+7.2*
P Symmetry	1	+4.3	+4.0
S.E.+		1.72	1.70
		Mean Squares	
Replicates	6	120	98.2
Lines	12	98.3	98.9
Families	164	132	134
Individuals	147	148	147

* P < 0.05, ** P < 0.01, otherwise P > 0.05.

Tests: See Footnote to Table 2.

+ Standard errors of a replicate mean, which are based on between-line variance.

females in the low compared to the high F lines which did not produce four litters either because of infertility (Table 3.3) or death before the end of the study (Tables 3.1 and 3.2) reduced the mean total production of young in the low lines more than in the high lines, creating a larger difference between the two than apparent from Method 1 and 2 analyses.

As with Method 2, the differences in litter size between the high and low A lines is relatively smaller and the difference between the high and low P lines relatively larger using Method 3 than when analysing the parities separately as with Method 1.

Although percent survival to the end of the study is slightly higher (+2.3%, P > Ø.5, Table 3.2) in the high compared to the low A lines, the difference is larger in the high compared to the low P lines (+4.2%, P > Ø.Ø5, Table 3.2). Judged over the 4 parities, the high A lines also have a 1.9% lower value of females producing litters than the low A lines, whereas the high P lines have a 1.9% advantage over the low P lines. These variations in survival and infertility would explain at least some of the variation in results from using the different methods of analysis for similar reasons as already stated for the F lines. In addition, the exclusion of records of animals that were humanely killed before the end of the study when Methods 2 and 3 analyses were used is a source of bias in the results of the high and low A lines; the animals humanely killed in the high A lines had been among the highest in the study for the total numbers of young produced up till the time of their deaths. Making the assumption that animals which were humanely killed would have performed as well on a fourth litter as in earlier litters had they been allowed to survive to the end of the study, then the inclusion of an adjusted record of their performance would have increased the differences between the high and low A lines by Ø.7 young for both measures of litter size.

Repeatability estimates for body weight of dams at mating, the

interval between pairing and birth, and litter size, either those born alive or the total born alive or dead are given in Table 3.11. As expected, B.W. has high repeatability whatever records the estimates are based on, however, P.B.I. has very low repeatability especially after mice have given birth to two litters. Using the addition of alive and dead young born as a measure of litter size (T.L.S.) gives higher repeatability estimates than using only the number of young born alive (A.L.S.). Using either measure of litter size, repeatability is highest between second and third litters although all the estimates made indicate that repeatability of litter size in mice is low relative to that of body weight.

3.4. DISCUSSION

The results show that changes in litter size in the lines selected for appetite and total lean mass evident at first litter are also present at the second, third and fourth litters. They also show that ovulation rate has changed much more than pre-natal survival to produce the alterations in litter size in these lines. In contrast, mice selected for percentage fat do not display significant changes in litter size at any parity studied, or in ovulation rate at fourth litter. As with the results of the study of first litters reported in Section 2, associated changes in body weight can explain the differences in litter sizes and ovulation rates in the P lines, but not all the differences in the A lines. S.C. Bishop (personal communication) investigated the carcass composition of mice from Generation 14 of the G strain, and has found large changes in percentage fat but no change in lean mass among the F lines, whereas there had been large changes in lean mass among the A lines and particularly among the P lines. This could explain the lack of any change in litter size and ovulation rate in the F lines if it is argued that lean mass is more closely related to ovulation rate than body weight (Fowler and Edwards, 1960; see also the discussion in Section 2).

TABLE 3.11. Repeatabilities (and confidence intervals) based on Parity 1 and 2, 2 and 3, 3 and 4, and Parities 1, 2, 3 and 4 for body weight of dams at mating (g) (B.W.), the pairing to birth interval (days) (P.B.I.), the number of young born alive (A.L.S.) and the total number of young born alive or dead (T.L.S.).

Repeatability based on	B.W.	P.B.I.	A.L.S.	T.L.S.
	Repeatabilities (and 99 percent confidence intervals)			
Parity 1 and 2	0.63 (0.491-0.730)	0.18 (0.029-0.330)*	0.19 (0 -0.368)	0.24 (0.050-0.415)
Parity 2 and 3	0.77 (0.679-0.839)	0.07 (0 - 0.225)*	0.35 (0.170-0.510)	0.36 (0.174-0.514)
Parity 3 and 4	0.68 (0.554-0.768)	0.09 (0 - 0.242)*	0.28 (0.087-0.446)	0.30 (0.133-0.466)
Parity 1, 2, 3 and 4	0.63 (0.515-0.724)	0.09 (0.004-0.184)*	0.26 (0.132-0.386)	0.27 (0.140-0.395)

* 95 percent confidence intervals.

Patterns of reproductive performance

A question of particular interest in this study has been whether the changes in first litter size in mice selected for appetite could have arisen because the peak of reproductive output in the high lines had shifted to an earlier time of life relative to that in the low lines. The results show that this is not the case, as the differences in litter size between the high and low A lines are similar in magnitude at the first two litters. The apparent decline in litter size of the high relative to the low A lines at third litter may have been partly caused by the likely presence of ringworm in the mice rather than being due to any shift in reproductive pattern as a consequence of selection. The high A lines would undoubtedly have had higher ovulation rates than the low A lines at third litter, given their 4.1 corpora lutea advantage over the low lines at fourth litter. This advantage may not have been fully expressed in terms of young born as it is conceivable that a disease such as ringworm could be detrimental to the survival of embryos and moreover the high lines, with their large potential for high litter size may have sustained a relatively higher pre-natal loss as a result of the disease than the low A lines. However, because of the time lag between replicates and the lack of disease-free contemporary mice, it is difficult to interpret the effects on litter size of the disease. In spite of the difficulties, there was an indication that mice had recovered from the effects of the disease by the fourth litter as the difference between the high and low selected A lines in foetus number (putative litter size) was larger than that for the number of young born at third litter.

The pattern of reproductive performance in all the selection lines agrees in general with that reported in unselected populations (Biggers et al., 1962; Rapp and Hedrich, 1974) where a peak of litter size is reached by the second or third parity with no significant decline noticeable until after the fourth litter. In this study however, a decline in post-implantation survival (a

component of litter size) is apparent when comparing the results of dissection at fourth parity with those of mice dissected at first litter reported in Section 2. The comparison is not ideal as 4 to 5 generations of selection had elapsed between sampling the mice for the two studies, but it may indicate trends. These trends are in agreement with a longer study in mice reported by Finn (1963) who found that the decline in litter size as animals aged was associated with a high level of embryo loss after implantation.

By the time of the fourth litter, chronological age or parity or both may have reduced pre-implantation survival in the high A and P lines compared to their contemporary low lines as the differences at first litter in this trait were much smaller (see Section 2). Biggers et al. (1962) suggest that pre-implantational and post-implantational failure both play a part in the decline of reproductive capacity with increasing maternal age or parity, and my study indicates that the decline may be more pronounced among lines where ovulation rate and litter size have changed as correlated responses to selection. Furthermore, the differences in pre-implantation survival recorded at fourth litter in the A and P lines were removed by fitting ovulation rate as covariate in the analyses, suggesting that maternal influences could be partly mediated by egg number, i.e. the higher the ovulation rate, the larger the decline in pre-implantation survival with maternal age or parity.

It needs to be pointed out that pre-implantation survival in this thesis refers to the combination of survival of the unfertilised egg and the pre-implantation embryo. However, several researchers have concluded that in the mouse, ovulation rate and embryo survival contribute much more to variation in litter size than fertilization rate (Bateman, 1958; Bradford, 1979). In mice super-ovulated with pregnant mare serum, more than 95 percent of the eggs recovered after natural mating were fertilized (Fowler and Edwards, 196Ø). It is therefore unlikely that pre-implantation survival results are very much influenced by variation in

fertilization rate in this study.

Survival of breeding females

The fact that selection for any of the criteria used in the present lines has not affected the ability of the breeding females to survive to the end of this study was unexpected in the case of mice selected for percentage fat. However, before making a general conclusion about the effects of fatness on survival per se based on these results, the following points need to be discussed. Firstly, how fat or lean were the mice? No objective measure of carcass fatness was attempted in this study. Interpretation of the results of carcass analysis would have been difficult because of the likely contribution pregnancy and variation in litter size would make to variation in fat reserves. In addition, knowledge of carcass composition in the F lines was already available from previous investigations, the differences in total fat percentage between the high and low lines measuring 78 percent of the control line mean in 17 week old males and females of Generation 14 stock (S. Bishop, personal communication). Subjectively, when mice were dissected at the end of my study, females of the high F lines appeared fatter than those of the low lines. This was very pronounced in the amount of fat surrounding the ovaries; the equivalent organs in the male being the ones which are surrounded by the fat depot under selection in the F lines, the gonadal fat pad. Taken as a whole, the evidence is that there are large differences in total fat percentage between the high and low F lines.

Secondly, mice were dissected between 217 to 234 days of age. How much longer would they be expected to live? Roberts (1961) reported a mean lifespan of 603 days (males and females) when averaged over various stocks of mice, with females surviving for a shorter time than males. Tomita et al. (1976) report a longer mean lifespan of 737 days, for females of the SPF C3H strain. It is obvious that my study, designed under the limits

of time and facilities, would not have provided a severe test of survival for mice in general, mean lifespan being about three times the age at dissection. The large differences in carcass composition which exist in the F lines could have altered mean lifespan drastically, but the length of study required to detect this would be longer than the one reported here. Thirdly, it may be that mouse survival depends more critically on a particular fat reserve of the body (or several) than on total fat percentage per se.

To conclude this section, fat percentage, lean growth and appetite, as defined by the selection criteria used in the G lines are apparently not genetically associated with the ability of breeding female mice to survive to 217 to 234 days of age.

Fertility

Selection for either appetite, total lean mass or fatness has not significantly influenced the ability of females in this study to produce litters from any particular mating period. However, there are two aspects to fertility (the ability to produce viable young), namely whether a female is fertile at any particular mating period, which may have some independence from what happens at another mating period and whether infertility occurs because the animal has stopped breeding altogether, i.e. that its reproductive life is over. My study did not provide a severe test in general for this latter aspect of fertility, as Roberts (1961) found that female mice in his trial stopped breeding at a mean age of about 300 days, (averaged

over several stocks). In Tomita et al.'s (1976) report of SPF C3H mice, the age of females at the birth of their last litter ranged from 128 to 352 days, with a mean of 261 days. Nevertheless, the lack of any change in fertility in the F lines is in apparent contradiction to the view that lines of mice selected for high body weight suffer infertility partly through their tendency to overfatness, especially at older ages (Roberts, 1965, 1979). Roberts (1961) found that such lines produced on average, only 4.5 litters under continuous breeding. Surely, if overfatness had been a main reason for infertility in the high body weight lines studied by Roberts and others (see Section 1), the lines selected for high fat percentage would have shown a decline in fertility by the fourth parity, but there is no suggestion of this in the results.

To conclude, fatness in the mouse appears not to be genetically associated with fertility when it is studied over four interval-bred litters. A more detailed discussion of possible reasons for the infertility experienced in lines of mice selected for increased body weight or gain is given in Section 6.

Pairing to birth intervals

The longer time it took in most cases for females of the control A and P lines to produce litters after pairing than either their contemporary high or low lines is difficult to explain. In the F lines, where the males used for mating were proven sires taken at random from unselected populations, there appeared to be no delay in arrival of litters in the control lines compared to the selected lines. This raises the possibility that the differences observed within the A and P lines could have been influenced by male libido, the male mates having come from the same lines as the females. There is no direct evidence for this however; the effect could have equally been caused entirely by a change in the components of female fertility among the selected A and P lines. A similar argument is also relevant to the observation that the high P lines took longer

to produce litters after pairing than the low P lines.

Although they are not entirely comparable, it is useful to consider what has been observed in other studies of selected populations. Falconer (1960) commented that in selecting for litter size, it took longer for litters to arrive after pairing in a control population than the selected line. In contrast, Fowler and Edwards (1960) found when investigating lines of mice selected for body weight that it was the selected lines where litters took longer to arrive after pairing than the control line. Male libido was found to be the primary cause of the delay in the line selected for high body weight whereas sterility of females was implicated in the line selected low body weight. The duration and intensity of selection and the level of inbreeding are likely to be important modifying factors when making these comparisons between studies. All that can be concluded from my study about the delay of the arrival of litters after pairing in the control A and P lines is that it is not a unique experience, nor are the differences large.

Post-natal maternal ability

The measures of post-natal maternal ability used of the entire weights of litters at 12 and 21 days of age were subject to error from several sources. An attempt to remove the error from variation in litter size was made firstly by adjusting litter size at birth to between 6 and 12 where possible, which was also done to conform with the selection experiment and, secondly, by fitting a curvilinear regression on litter size actually present at recording to the weights measured. Error from the pre-natal influences of foetal and placental size would have been adjusted for by fitting birth weight of the litter as a covariate in the analyses, but the adjustment was excluded as the regression failed to reach statistical significance ($P > 0.05$).

This relatively minor influence of pre-natal factors on the

results is in agreement with those of Falconer (1947) and Cox, Legates and Cockerham (1959) who found more than 70 percent of the variance in 12-day weights of standardised litters to be accounted for by post-natal influences. Furthermore, their reports suggest that the use of 12-day weight in a standardised litter is principally measuring the contribution of the dam's genotype, although Hanrahan and Eisen (1970) and Eisen (1974) caution against equating this entirely with lactational performance. Eisen (1974) suggests in his review that retrieving behavior and changes in maternal body weight (influencing maternal behaviour) may contribute to the variation in 12 day litter weight, independently of lactational performance. Although these complications are acknowledged, no attempt was made to objectively measure the individual components of the maternal contribution to the weights of litter recorded, nor was any account taken of the effect of the genotype of the offspring on the results.

A cross-fostering study like those reviewed by Eisen (1974) would have given a more accurate measure of maternal ability, but such a study was impractical as it would have interfered with the selection experiment and required too much technical work. Despite the weaknesses of the procedure actually used, the consistent and large advantage of the high A and P lines over their contemporary low lines in 12 and 21 day litter weights is evidence of their higher maternal ability. These differences are possibly associated with changes in maternal lean mass evident in the A and P lines; in the F lines, where no significant change in maternal body weight had occurred and probably none at all in lean mass (S. Bishop, personal communication), maternal ability did not vary between the high and low selections.

Long-term reproductive performance

Several workers have published accounts of so called 'lifetime' reproductive performance of mice from selected populations (Roberts,

1961; Wallinga and Bakker, 1978; Nagai et al., 198Ø, and von Butler et al., 1984). In most instances, the term 'lifetime' reproductive performance has meant the total accumulated number of young born or raised to weaning from continuously bred pairs of mice, i.e. one male and one female units, either until they die, or for a period close to the length of their reproductive life. Invariably, the conclusions drawn from these earlier studies have been different from those drawn from my study, as in their cases the reproductive lifespan of the animals was more fully expressed and became critical in determining the total number of young produced. This study has not aimed to measure 'lifetime' reproductive performance as such, but rather it has been designed within the limitations of time and resources to examine, firstly, whether the changes in first litter size in the A lines could be explained by an alteration in the pattern of reproductive output and, secondly, to examine that part of the reproductive life of a mouse which is appropriate to animal production. Few, if any efficient agricultural production systems retain animals until the end of their reproductive life, as their productive output by that time (e.g. the amount of milk, wool or offspring for slaughter) would be below that of younger animals, their sale value would be very low and their very presence until that time would increase the generation interval and reduce genetic progress.

Long-term reproductive rate in this study has been assessed by using Method 3 analysis, where litter size has been summed over parities, regardless of survival or fertility of the dam. It is clear that, when the mice are interval bred four times, total reproductive output in high A and P lines is higher than their contemporary low or control lines, in agreement with the differences found at first litter. A combination of slightly higher survival and fertility in the lines selected for high compared to low fat percentage give the high lines a small advantage in total reproductive output over the low lines, whereas there had been no difference at all in first litter size between the two selection

directions.

In conclusion, the changes in first litter size observed in the selection lines of the G strain are a reasonable indicator of the changes which occur in long-term reproductive rate measured over four interval-bred litters. First litter size may not be a reasonable indicator of lifetime reproductive rate if reproductive lifespan and longevity have altered as a consequence of selection, but this study which emphasises earlier reproduction, was not long enough to detect change in these traits. Finally, the connection between appetite and ovulation rate in the mouse is not dependent on the age or parity at which the females are studied, nor can it be explained fully by associated changes in body weight. The possible reasons for this relationship between appetite and ovulation rate are explored further in Section 4.

3.5. SUMMARY

Female reproductive performance based on 4 parities, together with survival of the dams and their post-natal maternal ability is reported in lines of mice previously selected for 13 generations for one of two criteria, either appetite (A), or total lean mass (P), and in other lines previously selected for 16 generations for percentage fat (F).

Female mice were first bred at 8 weeks of age, and thereafter at intervals of approximately 7 weeks until the fourth parity. Litters were adjusted to 6 and 12 young at birth and weaned at 21 days of age. In the fourth parity, breeding females were dissected in late pregnancy for measuring ovulation rate and the number of implants and live foetuses. The high A lines had higher litter sizes at the first three parities, and higher ovulation rates, implant and live foetus numbers at the fourth parity than the low A lines (high vs low line differences were +2.3, +2.2 and +Ø.9 total young born for parity 1, 2 and 3 respectively, and +4.1 corpora lutea, +1.8 implants and +1.4 live foetuses at parity 4). In the P

lines, the high lines were also higher than the low lines for the above traits (high vs low line differences were +2.2, +1.5 and +1.9 total young born for parity 1, 2 and 3 respectively, and +5.8 corpora lutea, +1.8 implants and +1.2 live foetuses at parity 4). In contrast, litter sizes and ovulation rates were similar in the high and low F lines.

Pre-implantation survival at the fourth parity was lower in the high A lines than in the low A lines (79.8 vs 88.Ø% respectively, $P > Ø.5$) and in the high compared to the low P lines (72.3 vs 85.7% respectively, $P < Ø.Ø5$). There were only small differences among the high and low selections of the A and P lines in post-implantation survival. No component of pre-natal survival differed substantially at fourth parity when the high and low F lines were compared.

Associated changes in body weight could explain, in general, all the differences in litter sizes and ovulation rates within the P lines, but not all the differences (particularly ovulation rates) in the A lines. It was thus confirmed that the large correlated changes which had occurred in ovulation rates and litter sizes at first parity in mice selected for appetite (described in Section 2) were associated with similar changes at later litters. This finding disproved the hypothesis that a shift in the timing of the peak of reproductive rate in the A lines could explain the changes observed at first litter.

There were only small differences in fertility at each parity and the percentages of females surviving to the end of the study when comparisons were made between the high and low selections of either the appetite, percentage fat or total lean mass lines. In the first three parities, dams of the high A lines produced heavier total weights of litters at 12 and 21 days after giving birth than dams of the low A lines (high vs low line differences for 12 day weights of +6.4, +8.6, +9.2g for Parity 1, 2 and 3 respectively, $P <$

Ø.5 for Parity 2 and 3). The comparable differences in the P lines were larger (high vs low line differences for 12 day weights of +12.9, +16.4 and +17.8g for Parity 1, 2 and 3 respectively, $P < Ø.Ø1$). There were no differences in these traits in the F lines.

When litter size, fertility and the ability of mice to survive were combined in the one trait (summing together performance at all parities), the average total number of young produced per female was significantly higher in the high compared to the low selections of the A lines (+5.Ø young born alive or dead, $P < Ø.Ø5$), the P lines (+7.2 young born alive or dead, $P < Ø.Ø5$), but not in the F lines (+2.Ø young born alive or dead, $P > Ø.Ø5$).

It was concluded that litter size at first parity in the A, F and P lines gave a reliable indication of reproductive rate during a major portion of the reproductive lifespan expected in mice, which in this study was taken as being 4 parities.

4. EFFECT OF SUPPRESSING THE HORMONE PROLACTIN IN THE MOUSE ON BODY WEIGHT, FOOD INTAKE AND OVULATION RATE

4.1. INTRODUCTION

In earlier sections of this thesis it has been reported from studies of previously selected lines that a relationship exists in the mouse between appetite and ovulation rate, and that the relationship held for a major portion of reproductive lifespan. In addition, the relationship described was much greater than that expected from changes in body weight.

The underlying physiological control of the relationship described is unknown, but it is likely to involve hormone action; for instance ovarian function is under the powerful influence of the gonadotrophins, which are secreted by the pituitary gland (Dickson, 1977). Any change in ovulation rate as a correlated response to selection will probably be mediated by a change in the secretory pattern of the gonadotrophins and/or modification of the ovarian sensitivity to their action. The question is, what has promoted these changes when selection has been practised for appetite?

There is evidence of differences in basal metabolic rate between the high and low appetite selections I have studied (S. Bishop, personal communication) and it is therefore possible that these changes are responsible for the changes in ovulation rate rather than appetite *per se*. From studies of wild populations, McNab (1980) argues that 'it behooves all mammals to have as high a rate of metabolism as can be sustained by the quantity and quality of their food resources in space and time, because this adjustment will permit them to maximize their reproductive efforts'. Hormones which can influence or be influenced by the appetite, growth and possibly the basal metabolic rate of a mammal and at the same time

influence the growth and development of the pre-ovulatory follicle are obvious candidates to examine when investigating the relationship described above. The hormone prolactin fits this category. Firstly, it appears to be involved in the growth process in mice (Sinha et al., 1972; Bohnet and Friesen, 1976; van Buul-Offers, 1984). Secondly, injections of prolactin rather than thyroid hormones result in increases in both appetite and growth rate in deer (Ryg and Jacobsen, 1982). More generally, there has been speculation about a role for prolactin (in conjunction with corticosteroids) in feeding behavior and growth in relation to the light-dark cycle, but this is still unclear (Brinklow and Forbes, 1984). Thirdly, there is evidence that prolactin is involved in follicular growth and development in a number of mammals (McNeilly, 1984). Although the sheep used were few in number, Rodway et al. (1983) reduced ovulation rate in anoestrous ewes at an induced oestrus by the long-term administration of bromocryptine , a drug which suppresses prolactin activity. Furthermore, Bohnet and Friesen (1976) suggest that lack of prolactin but not of growth hormone retards sexual development in dwarf mice as it may be important in the induction of luteinizing hormone receptors in the ovary.

This section describes an experiment which examines the role of the hormone prolactin in helping to mediate relationships between appetite and ovulation rate in the mouse. The method used has been to suppress endogenous prolactin activity and to examine the consequences of doing this on appetite (food intake), body weight and ovulation rate.

4.2. MATERIALS AND METHODS

The drugs used to suppress endogenous prolactin activity were bromocryptine (2-bromo-α-ergocryptine mesilate, CB 154, Sandoz Ltd., Basel, Switzerland) and cysteamine (2-mercaptoethylamine, Sigma, London). Both these drugs suppress circulating prolactin levels in

rats (bromocryptine, Gosden et al., 1981; cysteamine, Millard et al., 1982), and Yanai and Nagasawa (1970) have reported suppression of pituitary prolactin levels following bromocryptine administration in mice.

Preparation and use of drugs. Concentrated stock solutions of bromocryptine were prepared for each dose level used. The solvent used was 70% alcohol, slightly acidified with tartaric acid. Working concentrations for each dose level were made up daily by taking an aliquot of the stock solution and diluting it 6.67 times with 0.9% physiological saline. Cysteamine was dissolved in 0.9% physiological saline on the day of use. The appropriate quantity of either drug to be injected into each animal was administered subcutaneously in 0.1ml of vehicle.

As there were no published reports of systematic dose-response studies examining the effects of either bromocryptine or cysteamine on prolactin in mice, it was necessary to conduct dose-response trials before undertaking the main experiment.

Female mice used for the dose-response trials came from litters born to control populations of the G strain (Sharp et al., 1984). The litters in which they were born were adjusted downwards to 12 young at birth. At 3 weeks of age, the females were weaned and housed in stock cages with 4 to 6 mice per cage and placed in a room with a controlled daylength of 12 hours of light, in preparation for the injection period. From weaning onwards, the mice were fed No. 3 McGregors diet ad libitum, as were mice at similar ages used in the main experiment described below.

First dose-response trial. At 5.5 weeks of age, the females were weighed and allocated in harems of 3 to a male in mating cages. Vaginal plugs were used to indicate the day of mating. Females with vaginal plugs were randomly allocated to one of 9 treatments, either 0, 50, 100, 150 or 200ug of bromocryptine or 1.5, 3, 6 or 9

mg of cysteamine. The zero dose treatment used was 0.9% physiological saline, the same as that used to dissolve cysteamine.

Twice-daily subcutaneous injections were given to the mice, from the evening of Day 5 of pregnancy, until the morning of Day 9. (Day 0 = day of vaginal plug). Four to five hours after the last injection, animals were rapidly decapitated, the trunk blood being collected via a heparinized glass funnel into a glass tube. Blood samples were centrifuged at 3500rpm for 20 minutes at 4°C and the plasma recovered and stored at -20°C awaiting radioimmunoassay.

Once the blood sampling had been completed, reproductive tracts were examined for evidence of pregnancy and embryonic resorption. This provided a bioassay for the effectiveness of the dose of drugs in reducing prolactin activity, as the hormone is important in the maintenance of early pregnancy (Choudary and Greenwald, 1969; Bartke, 1973).

Second dose-response trial. At 5.5 weeks of age, 40 females were weighed and randomly allocated within litters to 4 treatments; where possible each group contained one female from each litter. The treatments were 0, 50, 100 and 150ug of bromocryptine injected twice-daily, at 0830 and 1930 hours, for 2 days. The zero dose was the vehicle used to dissolve the drug. Mice were rapidly decapitated 7 to 7.5 hours after the last injection, blood samples being collected, centrifuged, processed and stored as previously described. By introducing a delay in the start of injections for some mice relative to others, it was only necessary to decapitate ten animals on any one day. This was arranged to minimise the release of prolactin due to disturbance stress (Döhler et al., 1977).

Main Experiment

Mice and management. Mice from replicate 1 of generations 16 and 17 of the high appetite selection lines (Sharp et al., 1984) were mated to produce offspring for use in this experiment. Litters were adjusted at birth to between 8 and 12 young. At weaning the young virgin females were housed in stock cages with 4 to 6 per cage and placed into a room with a controlled daylength of 12 hours of light, in preparation for the injection regime.

At six weeks of age, the female mice were weighed and housed individually, in cages designed to measure feed intake. After a 4 day period to allow for adjustment to their new surroundings, the animals were weighed again and allocated at random within litters to one of four treatments, either 0, 50, 100 or 150ug doses of bromocryptine given twice daily, starting at 0830 and 1930 hours. The zero dose was the vehicle used to dissolve the drug. During the following 12 days of the injection regime, the mice were weighed every second day, and the food consumed was measured every 4 days. On the 12th day of injections, the mice were moved to metal cages with 4 individuals to each cage. This was done to minimise the number of males required for mating, and to create an environment where disturbance stress at sacrifice would be lower than if the mice had remained individually housed in the plastic cages designed for measuring food intake; these cages gave mice a clear view of the surrounding area.

The female mice continued receiving twice-daily injections of the same quantities of bromocryptine, as before. After they had spent two days in the metal cages, a male was introduced. Daily checks were made for vaginal plugs, and the female mice were rapidly decapitated two hours after the injection given during the morning of the day a plug was found. Blood samples were collected, centrifuged, processed and stored as previously described, awaiting radioimmunoassay. Ovulation rate was determined by firstly locating and freeing the cumulus with embedded ova from its position in the fallopian tube. Once freed, the cumulus was picked up with a pasteur

pipette and deposited on a microscope slide, covered with a cover slip and the number of ova it contained counted using a binocular dissecting microscope.

Radioimmunoassay of prolactin. Materials for assay of mouse prolactin were obtained from NIADDK, Maryland, U.S.A. After validation of the procedure (see Section 5), samples were assayed in duplicate, using 25ul aliquots of plasma. A separate assay run was used for each of the dose-response trials; a third assay run was used for the main experiment. Comparisons of samples from different assay runs are subject to inter-assay run variation; the mean concentrations of the quality control standards in the 3 assay runs performed were 177.2, 217.2 and 220.5ng/ml for the high quality control and 38.4, 37.4 and 33.1ng/ml for the low quality control, respectively. Based on 7 assay runs, the mean concentration for the high quality control was 213.3 ng/ml, with a coefficient of variation of 14.4%. The minimum detectable dose was 0.16ng/assay tube, which is equivalent to 6.4ng/ml when 25ul of plasma are used.

4.3. RESULTS

First dose-response trial. The results of this trial are given in Table 4.1. All the females used had mated, as shown by the presence of a vaginal plug, and embryos were presumed to have implanted by the start of the injection regimes. Twice-daily doses of either 3.0mg of cysteamine or 100ug of bromocryptine were enough to cause a significant decline in the frequency of mice with live embryos compared to the control group of mice injected with vehicle only. (Chi-square analysis used).

Prolactin concentrations in the plasma of mice injected with any of the doses of cysteamine or bromocryptine used were not lower than those of mice of the control group, and in the case of the 9.0mg dose of cysteamine the concentration was significantly higher ($P < 0.01$, using the Wilcoxon Rank Test). This failure to

TABLE 4.1. Effects of dose of cysteamine (CHS) and bromocryptine (CB154) on plasma prolactin concentration and on the number of mice with and without live embryos in the first dose-response trial.

	Number of Mice			Prolactin in plasma (ng/ml)	
Treatment	Total	Pregnant	Not pregnant	Median	Range
Control	8	7	1	12.5	6.3-29.2
1.5mg CHS	7	5	2	10.5	5.6-13.4
3.0mg CHS	7	2*	5	13.8	7.5-29.5
6.0mg CHS	7	2*	5	33.6	7.6-58.3
9.0mg CHS	6	0*	6	21.5*	16.4-62.7
50ug CB 154	7	6	1	12.5	11.1-72-8
100ug CB 154	7	2*	5	14.0	9.5-17.9
150ug CB 154	7	2*	5	13.4	9.9-22.4
200ug CB 154	7	1*	6	14.6	11.4-102.5

*$p < 0.01$ compared to control values.

(Using X^2 analysis for the numbers of animals with and without live embryos and Wilcoxon's Test for rank of prolactin concentrations).

demonstrate suppression of prolactin made it necessary to conduct a second dose-response trial with virgin mice. Complications would also have arisen in deciding the correct doses for virgin mice (used in the main experiment) based solely on information gained from mated female mice, because of doubt that the two classes of mice could have been compared directly.

Second dose-response trial. The results of this trial are given in Table 4.2. As cysteamine injection made the mice very unhealthy in the first dose-response trial (one animal died in the group receiving the highest dose), the second trial was conducted with bromocryptine only.

All dose levels significantly reduced the prolactin concentrations in plasma of the virgin female mice when compared to those in the control group receiving the drug vehicle only. However, relative to each other, the 50, 100 and 150 ug dose levels gave similar suppression of plasma prolactin concentration.

Shire (1976) regards plasma concentrations as unreliable reflections of hormone activity at the target organs. In view of Shire's opinion, the finding that it required a 100ug dose of CB154 to detect interruption of pregnancy in mated female mice (the first dose-response trial) was regarded as relevant information in the choice of appropriate doses.

The results therefore of the two dose-response trials led to the choice of 0, 50, 100 and 150ug doses of bromocryptine for the main experiment.

Main Experiment

Results of food intake, ovulation rate and plasma prolactin concentration are given in Table 4.3. Figure 4.1 displays the body weight of the mice during the 12 day period of treatment when their

TABLE 4.2. Effects of dose of bromocryptine (CB 154) on plasma prolactin concentration in the virgin mice used in the second dose-response trial.

Treatment	Number of Animals	Prolactin in plasma (ng/ml) Median	Range
Control	10	25.6	21.0-76.2
50ug CB 154	10	11.7**	10.5-20.0
100ug CB 154	10	15.0*	9.8-31.1
150ug CB 154	10	14.1**	11.2-36.1

* $P < 0.05$ ** $P < 0.01$, when compared to control mice, using the Wilcoxon Rank Test.

TABLE 4.3. Least-square means from analysis of variance for the amount of food eaten per mouse before and during the injection period, ovulation rate, and median and range of plasma prolactin level following administration of bromocryptine (CB154)

Treatment	No.per group	Food eaten in the 4 days before injections (g)	Food eaten during injection period (g)				Ovulation Rate	Plasma prolactin level (ng/ml)	
			Day 1 - 4	Day 5 - 8	Day 9 -12	Day 5 -12		Median	Range
Control	13	20.3^{a}*	22.5^{a}	21.9^{a}	22.2^{a}	44.1^{a}	14.9^{a}	217.2^{a}	30.3 - 366.2
50µg CB 154	14	19.8^{a}	21.5^{a}	22.9^{ab}	24.3^{ab}	47.2^{ab}	14.4^{a}	32.9^{b+}	16.3 - 150.5
100µg CB 154	14	20.2^{a}	21.8^{a}	23.4^{ab}	25.0^{b}	48.4^{b}	15.1^{a}	32.5^{b+}	15.5 - 161.3
150µg CB 154	13	20.2^{a}	21.1^{a}	24.3^{b}	26.0^{b}	50.3^{b+}	15.7^{a}	31.6^{b+}	16.1 - 121.1
s.e.‡		0.74	0.71	0.72	0.84	1.50	0.60		

* Means or medians within columns with unlike superscripts differ significantly ($P < 0.05$) (Pair-wise comparisons used for food eaten and ovulation rate and for plasma prolactin level where Wilcoxon's Rank Test was used).

\+ $P < 0.01$ compared to control.

‡ Standard errors of a treatment mean based on residual within-treatment variance.

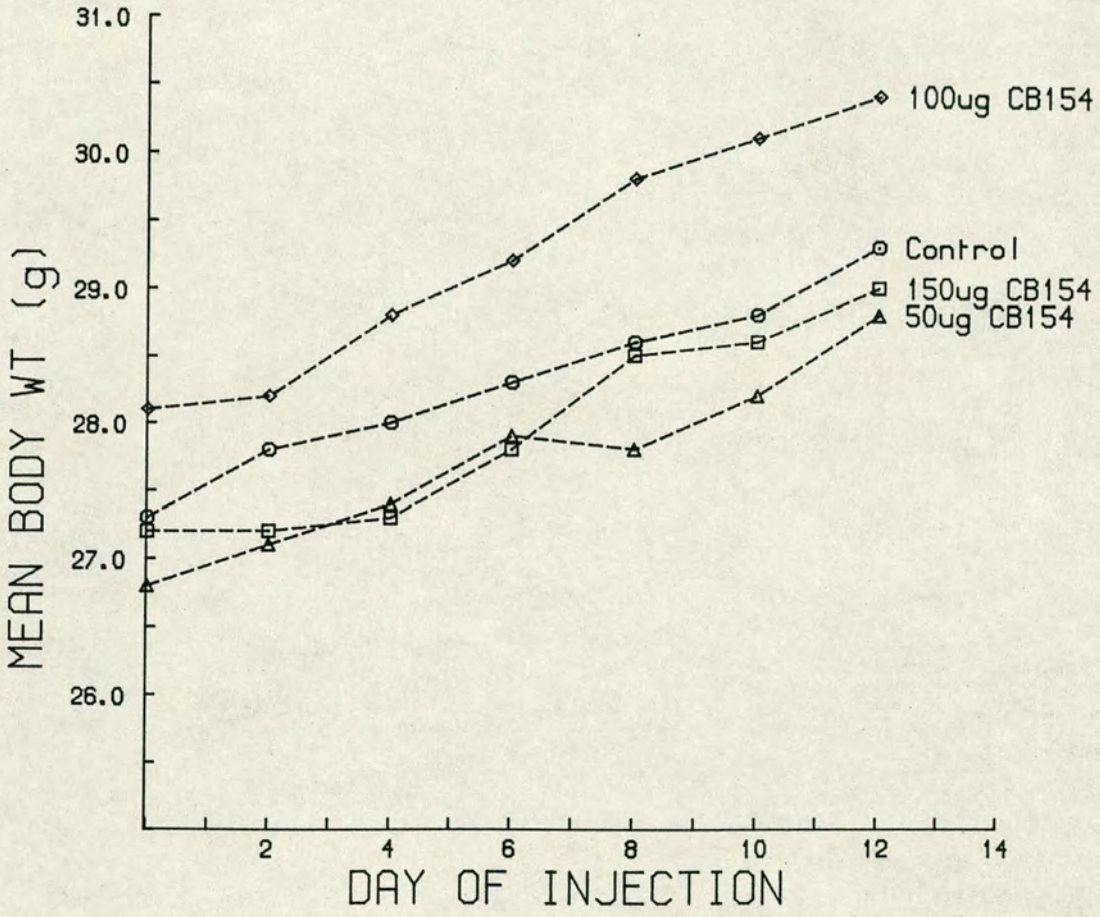


FIGURE 4.1. Least-square means from analysis of variance of body weight of the mice during the period they received bromocryptine (CB154) injections. The slope of each line is 0.16, 0.15, 0.21 and 0.16g/day for the mice receiving Ø (Control), 50, 100 and 150µg CB154 respectively, with no significant difference among slopes ($P > 0.05$). Typical standard error of a slope= 0.040g/day.

food intakes were recorded, and also gives the growth rates they achieved. Except for hormone concentration, all the traits had been subjected to analysis of variance by least-squares.

All dose levels of bromocryptine used significantly reduced the prolactin concentrations in plasma of the mice when compared to the control animals receiving vehicle only, with each dose giving similar suppression of plasma hormone activity. This confirmed the findings of the second dose-response trial. Ovulation rates were not affected by any dose of bromocryptine administered. Animals receiving 100ug of bromocryptine grew 0.06g/day faster than mice in the other treatment groups, but this difference was not significant ($P > 0.05$).

After an initial 4-day adjustment period, the higher the dose of bromocryptine given, the larger the quantity of food consumed by the mice, the dose-response relationship being almost linear. From day 5 to 12 of treatment, individuals receiving either 50, 100 or 150ug of bromocryptine, ate 3.1g, 4.3g and 6.2g more food, respectively, than mice in the control group.

From a subjective examination of the mice cages, no evidence could be found of extra food wastage among animals receiving higher doses of bromocryptine; food wastage therefore is not likely to be an important source of error in the food intake measurements.

4.4. DISCUSSION

The results do not support the hypothesis that prolactin directly mediates a relationship between appetite and ovulation rate in the mouse. If the hypothesis had been correct, changes in ovarian activity would have accompanied the drug-induced suppression of prolactin concentrations in the animals which received injections of bromocryptine. Clearly, this was not the case, as ovulation rate

was unaffected by any dose of CB154 given.

This finding is in apparent contradiction to the suggestion that prolactin plays a role in inhibiting oocyte maturation (Baker and Hunter, 1978). It is also at odds with a recent review; McNeilly (1984) concluded that evidence from a number of mammalian species indicated a role for prolactin at the ovarian level in influencing follicular growth and development.

Whereas prolactin is involved in the maintenance of the corpora lutea of pregnancy and pseudopregnancy in the mouse (Dominic, 1966; Choudary and Greenwald, 1969; Bartke, 1973), its exact involvement in pre-ovulatory events in any species remains unclear. Prolactin may indeed affect the quality of oocytes destined to ovulate without influencing the number that do. Alternatively, the concentration of prolactin may influence ovulation rate, but only after a long period, longer than the period that prolactin levels were suppressed in this study. Suppressing prolactin concentrations in anoestrous ewes by injecting bromocryptine for 87 days reduced the average number of corpora lutea at an induced oestrus when compared to counts made in a control group (Rodway et al., 1983). This evidence however was based on the records of only six treated and six untreated ewes, so chance (despite statistical significance) or factors other than the bromocryptine administration (e.g. variation between treated groups in response to the oestrous induction method, in particular, to the use of pregnant mare serum) could have had a large influence on the results.

On the other hand, it is known in mice that injections of pregnant mare serum gonadotrophin given 43 hours before oestrous induction treatment can markedly influence ovulation rate (Fowler and Edwards, 1960; Land, 1965), suggesting that its final determination occurs within the preceding oestrous cycle. As the

minimum duration of bromocryptine administration in my study was 15 days for any individual mouse, there would appear to have been plenty of time for the lowered prolactin concentrations to influence whatever factors are critical in the final determination of ovulation rate.

It needs to be pointed out that bromocryptine could influence other hormones independently of its effect via pituitary prolactin release, for instance the drug is a dopamine receptor agonist (Mehta and Tolis, 1979) and dopamine itself may inhibit gonadotrophin secretion (Evans et al., 1982), although the evidence for this is not conclusive (Sirinathsinghji and Martini, 1984). In rats, bromocryptine suppressed the LH release induced by synthetic GnRH or rat hypothalamic extract, but did not alter serum FSH levels (Seki et al., 1974). Recent evidence indicates that the drug can modulate pituitary GnRH receptors independently of changes in serum prolactin, although LH and FSH concentrations in plasma did not show any effects of treatment (Clayton and Bailey, 1984). Despite these possibilities, ovulation rate was unaffected by any dose of bromocryptine given in the main experiment.

In addition to measuring ovarian activity, food intake was also recorded to see whether it had been affected by prolactin suppression. However, to find evidence in favour of the hypothesis, it was not necessary to find alterations in appetite; appetite itself could have been involved in determining prolactin activity, rather than vice-versa.

In the event, the suppression of prolactin in the main experiment was associated with increases rather than decreases in food intake. This was contrary to expectation, as Ryg and Jacobsen (1982) have shown that injections of prolactin increase appetite in deer. In addition, food intakes and growth rates of sheep, cattle and deer are higher in summer months, a time of the year when prolactin activity is also high; proof is however still lacking

that prolactin is causally involved in the increased growth and appetite observed in these animals in response to long daylength (Forbes, 1982).

There are a number of possible explanations for the responses in food intake to bromocryptine administration in the main experiment. Firstly, the physiology of the mouse may be somewhat different than that of other mammalia. Secondly, a more likely explanation is that the action of bromocryptine is not restricted to suppressing prolactin release from the pituitary gland. The drug also has effects on the hypothalamus, the central and peripheral nervous systems and the gastrointestinal tract (Flückiger, 1976 and review by Mehta and Tolis, 1979), one or more of which could be important in appetite control. For instance, when bromocryptine is administered to rats, dopamine turnover is slowed in the arcuate nucleus of the hypothalamus (Hökelt and Fuxe, 1972). This could either be a direct effect of the drug or a more indirect one through the involvement of dopamine in mediating the ability of prolactin to modify its own secretion, the so-called 'short loop' feedback effect (Höfelt and Fuxe, 1972; Advis et al., 1977; Gudelsky and Porter, 1980; Whitworth, Grosvenor and Mena, 1981; Sarkar, Miki and Meites, 1983). Whatever the precise method of drug action, dopamine is an important catecholamine, and when injected into the lateral hypothalamus of hungry rats, it has been shown to suppress feeding behaviour (Leibowitz, 1976). An alteration in dopamine turnover from bromocryptine administration therefore might affect satiety in mice, which could explain the results of this study, but would still be in apparent conflict with the work in deer (Ryg and Jacobsen, 1982). One can not ignore other possibilities however; bromocryptine has been observed to increase spontaneous motor activity in mice in a dose-dependent fashion (dose range 2.5 to 10ug/g of body weight, Flückiger, 1976), and it can also induce hypothermia in mice (Flückiger, 1976) and rats (Calne et al., 1975), albeit at high dose levels (5-20ug/g of body weight, Calne et al., 1975). There are also dopamine receptors in

the gut, which probably mediate the inhibitory effect of dopamine on gastric emptying in man (Harrington et al., 1983). These are all additional sites and mechanisms by which bromocryptine could have acted to influence appetite in the treated mice. The actual mechanism or mechanisms of drug action therefore remain obscure.

As pregnant mice generally have lower prolactin levels than non-pregnant mice (Muir, Bradford and Geschwind, 1974; Sinha, Salocks and Vandeerlaan, 1975), the interruption of pregnancy in the groups receiving the higher doses of either bromocryptine or cysteamine during the first dose-response trial may have jeopardised the opportunity to demonstrate differences in plasma prolactin activity due to treatment. It is suggested that there were two opposing influences on hormone activity - the first because prolactin concentrations were attempting to return to levels found in non-pregnant animals and the second a depressing effect from the administration of bromocryptine. Detecting suppression of prolactin activity in the groups receiving drugs in the first dose-response trial was also made difficult by the already low concentrations in the control group. Despite these problems, the use of the presence or absence of signs of maintenance of pregnancy as a bioassay provided information in addition to the evidence of suppression of plasma prolactin concentrations obtained from the second dose-response trial, as plasma concentrations are not likely to give a perfect reflection of hormone activity at the target organs (Shire, 1976).

The design of the second dose-response trial had to cope with the knowledge that prolactin concentrations do vary with the time of the oestrous cycle. I decided to ignore its effect on the results for the following reasons. Firstly, it did not contribute a significant amount of variation to the results of a previous study with mice (Sinha et al., 1975). Secondly, the magnitude of the variation in prolactin concentrations due to the time of the oestrous cycle is far less than variation from comparisons between

individual mice (Sinha et al., 1972a and b, 1975). By not having to measure time of the oestrous cycle (a time-consuming process) a larger number of mice were able to be incorporated in the trial, thus reducing the chance of between animal variation masking the effect of dose of bromocryptine on hormone concentrations.

The much higher prolactin concentrations in the control group of the main experiment than in samples from similarly untreated mice in the dose-response trials could be mostly due to the surge of prolactin experienced in female mice following mating (Bartke, 1973); the mice in the main experiment having been blood sampled on the morning of finding a vaginal plug. It could have also been partly due to the shorter amount of time available in the main experiment than in the dose-response trials for the extra prolactin released following the stress of injection to have dispersed (2 hours compared to 4 to 7 hours, respectively). This possibility could not be avoided by delaying decapitation in the main experiment, as this would have made an accurate count of ova much more difficult, since the cumulus cells in which the ova were embedded would have dispersed throughout the fallopian tubes before the completion of measurement (Land, 1965).

The failure of any dose of bromocryptine to significantly influence body growth of the mice agrees in part with a report of Yanai and Nagasawi (1970), who found no effect of the drug on body weight in 7 to 8 month-old female mice. In contrast, although suppression of prolactin concentration in lactating mice with mouse prolactin antisera did not affect body growth at the lower of the two doses used, at the higher dose, weight gain was abolished (Sinha et al., 1972a). This finding is supported by the small increases in growth rate found when exogenous ovine prolactin is given to Snell dwarf mice (Wallis and Dew, 1973; Bohnet and Friesen, 1976; van Buul-Offers, 1984), which are deficient in growth hormone and prolactin activity (Bartke, 1967). Considering that the methods of manipulating prolactin, the level of suppression or augmentation

achieved and the experimental animals used differed between the cited reports and this study, the apparent conflict in results is not serious.

Finally, the post-pubertal mouse was used as a model in this experiment, as pre-pubertal animals may have failed to become sexually mature under bromocryptine administration (Advis, Smith-White and Ojeda, 1981), in which case natural ovulation rate could not have been measured. However, the relationship discovered between appetite and ovulation rate arose from mice selected for the amount of food eaten between 4 to 6 weeks of age (adjusted phenotypically for 4 week body weight), a period during which the females are going through the onset and attainment of puberty, the males being slightly later. A different physiological background may exist during this neo-pubertal period than afterwards, and prolactin could play a more important role at this time than the one found in the post-pubertal mice used in this experiment.

4.5 SUMMARY

This section describes the results of an experiment which examined if the hormone prolactin is involved in mediating relationships between appetite and ovulation rate in the mouse. The method used was to suppress endogenous prolactin activity with injections of bromocryptine (CB154) and examine the consequences of doing this on appetite (food intake), body weight and ovulation rate. Preliminary dose-response trials were conducted to choose appropriate amounts of drug to use for hormone suppression in the main experiment. Based on this evidence, dose treatments of Ø, 5Ø, 1ØØ and 15Øug of CB154 injected twice-daily were given to 46 day old virgin mice (there were 13, 14, 14 and 13 mice in the groups, respectively) for a minimum of 15 days. All studies were performed under controlled lighting conditions, with 12 hours of light per day. After an initial 4-day adjustment period, the higher the dose

of CB154 given, the higher was the quantity of food consumed by the mice, the dose-response relationship being almost linear. From day 5 to 12 of treatment, individuals receiving either 50, 100 or 150ug of CB154 consumed 3.1g, 4.3g and 6.2g more food, respectively, than mice in the control group. Body weight and gain however were unaffected by injection of CB154. From day 0 to 12 of treatment mice grew 0.16, 0.15, 0.21 and 0.16g/day in the 0 (control), 50, 100 and 150ug CB154 groups, respectively, ($P > 0.05$). Fourteen days after starting the injections, males were introduced and females were decapitated to obtain blood samples on the day they mated. Counts were also made of ova embedded in cumulus cells. Prolactin concentrations were suppressed, but ovulation rates were similar in the 50, 100 and 150ug CB154 groups compared to the control (median prolactin concentrations and mean ovulation rates were 32.9 and 14.4, 32.5 and 15.1 and 31.6ng/ml and 15.7 ova, respectively, compared to 217.2ng/ml and 14.9 ova in the control).

The results did not support the hypothesis that the hormone prolactin directly mediates a relationship between appetite and ovulation rate in the post-pubertal mouse.

5. THE PROCEDURE AND VALIDATION OF A METHOD FOR MEASURING MOUSE PROLACTIN

5.1. INTRODUCTION

The experiment reported in Section 4 required a means of measuring plasma prolactin concentrations in the mouse. As a preliminary test, non-experimental samples of mouse serum were subjected to radioimmunoassay with a procedure utilizing antiserum raised against rat prolactin. This method has been used before for the mouse by Cattanach et al. (1977). It was also convenient to try as the necessary materials and skills were available through the co-operation of Dr Alan McNeilly, of the Centre for Reproductive Biology in Edinburgh. Although concentrations in the physiological range were obtained, the method had poor precision since there is only partial immunological identity between rat and mouse prolactin (Sinha et al., 1972b); it was therefore deemed inappropriate. Procedures utilizing antisera raised against prolactins of various species other than the mouse and the rat would have been quite inappropriate to use because of their very low cross-reactivity when measured in a homologous radioimmunoassay for the mouse hormone (Sinha et al., 1972b).

For precise measurement of mouse prolactin concentrations in plasma, it was therefore necessary to use a radioimmunoassay based on antiserum raised against mouse prolactin.

A description of such a radioimmunoassay and the steps taken to validate the procedure used are given in this section.

5.2. MATERIALS AND METHODS

The materials for the radioimmunoassay of mouse prolactin were kindly provided by the National Hormone and Pituitary Program of the

NIADDK, National Institutes of Health, Bethesda, Maryland, U.S.A.

Mouse prolactin (mPRL) Highly purified mPRL (NIADDK - mouse PRL-AFP-4111-E) was used for iodination and for preparation of standards. Multiple aliquots of mPRL dissolved in 0.01 M $NaHCO_3$, at a concentration of 100ug per ml were prepared and stored at -70°C for iodinations. Mouse prolactin for use as standard was prepared at two concentrations, 10ug per ml and lug per ml, by dissolving it in 0.1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.5 (subsequently used as the general assay diluent). Aliquots of the 10ug per ml and the lug mPRL per ml solutions were stored at -70°C until use.

Antiserum. The mPRL antiserum AFP-131078 (NIADDK) which had been raised in rabbits was supplied already dissolved in 2% normal rabbit serum at a 1:50 dilution. It was further diluted in 0.1% BSA in PBS to 1:500. Multiple aliquots of the 1:500 dilution were prepared and stored at -70°C until use.

Second antibody (Donkey anti-rabbit gamma globulin) was supplied by the Scottish Antibody Production Unit.

Iodination of mouse prolactin. A modification of the method of Morrison and Boyse (1970) was used. Before starting iodination, 10ul of 0.5M phosphate buffer of pH 7.5 was added to one of the vials containing 2.5ug mPRL which had previously been dissolved in 25ul of 0.01M $NaHCO_3$. One mCi of ^{125}I as sodium iodide (Amersham) was added to the 2.5ug mPRL followed by 0.5mg of lactoperoxidase (in 10ul of 0.1% BSA in PBS) and 10ul of 0.01% hydrogen peroxide in distilled water. The reaction was stopped after 120 seconds by adding 250ul of 0.1% BSA in PBS. In the first two iodinations performed the mixture was transferred immediately to a column (1 x 20cm) of Sephadex G-100, which had previously been equilibrated with 4% BSA in PBS. The residue in the vial was washed with a further 250ul of 0.1% BSA in PBS and also transferred to the column. The

eluate from the column was collected in fractions of 1ml and counted for radioactivity using a Minimonitor. Material corresponding to the leading edge of the peak of radioactivity and a second pool of material corresponding to the trailing edge of the peak were retained from the first iodination for use in assays. Following the second iodination, only the material from the leading edge of the peak of radioactivity was collected.

To improve the percent of radioactively-labelled mPRL that would bind to antiserum during assays, the chromatography procedure was altered for the third iodination. The mPRL, after it had reacted with ^{125}I was transferred initially to a Sephadex G-50 column (1 x 20cm). Two separate pools of material were collected, the first corresponding to the leading edge (Pool 1) and the second to the trailing edge (Pool 2) of the peak of radioactivity. These two pools were kept apart and transferred to separate Sephadex G-100 columns. From each column, material corresponding to the leading and trailing edges of the peak of radioactivity were retained (Label A and B, respectively, from the column which contained Pool 1, and Label C and D, respectively, from the column which contained Pool 2) and all were checked for binding. Label D had the highest binding and was used in assays. In subsequent iodinations, only one pool of material corresponding to the peak of radioactivity from the eluate of the Sephadex G-50 column was purified further on a Sephadex G-100 column, but otherwise the procedure was similar to that during the third iodination.

Assay procedure. All dilutions were made in PBS, pH 7.5 containing 0.1% BSA. A range of concentrations of standard mPRL were prepared, in triplicate, by withdrawing various volumes from the 0.5ml of diluent already dispensed into each tube and replacing it with the same volume of a solution containing 20ng/ml of the standard mPRL. Twenty-five to 35 ul of the unknown samples, made up to a volume of 0.5ml with diluent were also dispensed into tubes in duplicate. One hundred ul of ^{125}I mPRL, approximate specific

activity 120uCi/ug, containing about 12,000cpm were added to each tube containing either standard or unknown sample, followed by 200ul of diluted mPRL antiserum. This made the total volume in each tube 0.8ml. A 1:320,000 dilution of the antiserum usually bound between 22 to 30% of labelled mPRL in the absence of competing unlabelled mPRL. The mixture was incubated at 4°C for 24hr, then 100ul of a 1:200 dilution of normal rabbit serum followed by 200ul of a mixture of a 1:40 dilution of donkey anti-rabbit serum and a 1:10 dilution of 0.1M EDTA were added to each tube. The tubes were incubated for another 24hr at 4°C. One ml of diluent was then pipetted into each tube, the tubes were centrifuged at 3800rpm for 25min and the supernatant was discarded. The precipitate was counted in a gamma counter (LKB Riagamma).

The counts obtained from the gamma counter were analysed using the ABRO data processing program package. In brief, this calculates a calibration curve for bound/free hormone ratios, by applying a logit-log transformation and a regression analysis to each batch of standards and to the overall standards incorporated into every assay. Potency estimates (estimated concentration of prolactin) are calculated for each unknown sample, by relating the counts obtained to the calibration curve.

Validation of the assay. The specificity of the antiserum had already been established by Dr A.F. Parlow of the Pituitary Hormones Center, Harbor/UCLA Medical Center, Torrance, California, U.S.A. The specificity of the method of radioimmunoassay used for mouse prolactin has also been reported by Sinha et al. (1972b). A range of concentrations of antiserum to mPRL, donkey anti-rabbit gamma globulin and the normal rabbit serum were tested so that their concentrations could be optimised before using the assay for determining concentrations of hormone in unknown samples. To demonstrate parallelism of immunoreactivity between standard mPRL and endogenous mouse PRL in assay samples, serial dilutions of mouse plasma were tested. To test for accuracy, recovery of mPRL added

at several concentrations to pooled mouse plasma was determined. To verify precision, two controls of pooled mouse plasma were included in each assay. These consisted of a pool of plasma from decapitated mice that had been given bromocryptine injections ('low' quality control) and a pool of plasma from mice bled directly from the heart after being anaethesatised with ether ('high' quality control).

5.3. RESULTS

Iodination. Figure 5.1 shows a typical elution profile of radioiodinated mPRL. On the Sephadex G-50 column, the sharply defined peak, in fractions 7 and 8, contained the labelled protein used. The small second peak, in fractions 14 to 18 was the free iodide. When an aliquot of the first peak was passed through a Sephadex G-100 column, only one peak emerged, and this contained the labelled protein. Material from the trailing edge of this peak was used in assays as it was more immunoractive than material from the leading edge of the peak.

Assay characteristics. Figure 5.2 shows a typical standard curve for mPRL in the range of 0 - 10ng. The concentrations on the abscissa refer to the amount of prolactin in an assay tube. Sensitivity of the assay, defined as the sum of two standard deviations away from the mean of the zero standard (i.e. no unlabelled hormone added) was 0.16ng/tube (average of 3 assays). If 80ul of a plasma sample are used for the assay, this system can detect as little as 2ng of mPRL/ml of plasma.

The dilution-response curves for normal mouse plasma (samples taken by decapitation), plasma from mice under ether stress (high quality control) and plasma from decapitated mice following a period of daily injections of bromocryptine are shown in Figure 5.3. The three curves appeared parallel to the standard mPRL curve (which is also shown), suggesting immunological identity between the standard

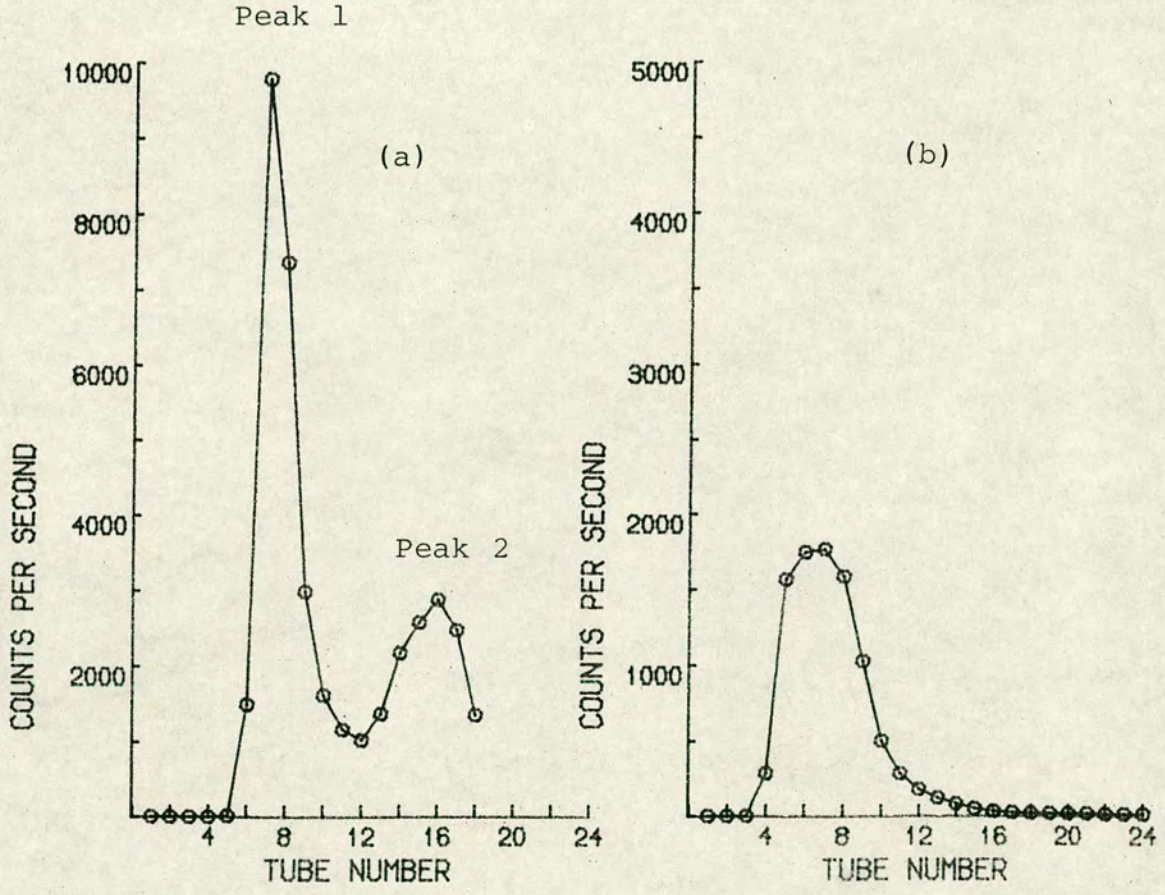


FIGURE 5.1. Elution profile of radioiodinated mouse prolactin from (a) a Sephadex G-50 column, with 0.5ml/tube, (b) a Sephadex G-100 column, with 1 ml/tube. Counts per second for each fraction of eluate collected are plotted against the number of the tube in which the fraction was contained. For the Sephadex G-50 column, Peak 1 contained the labelled hormone whereas Peak 2 contained the free iodide.

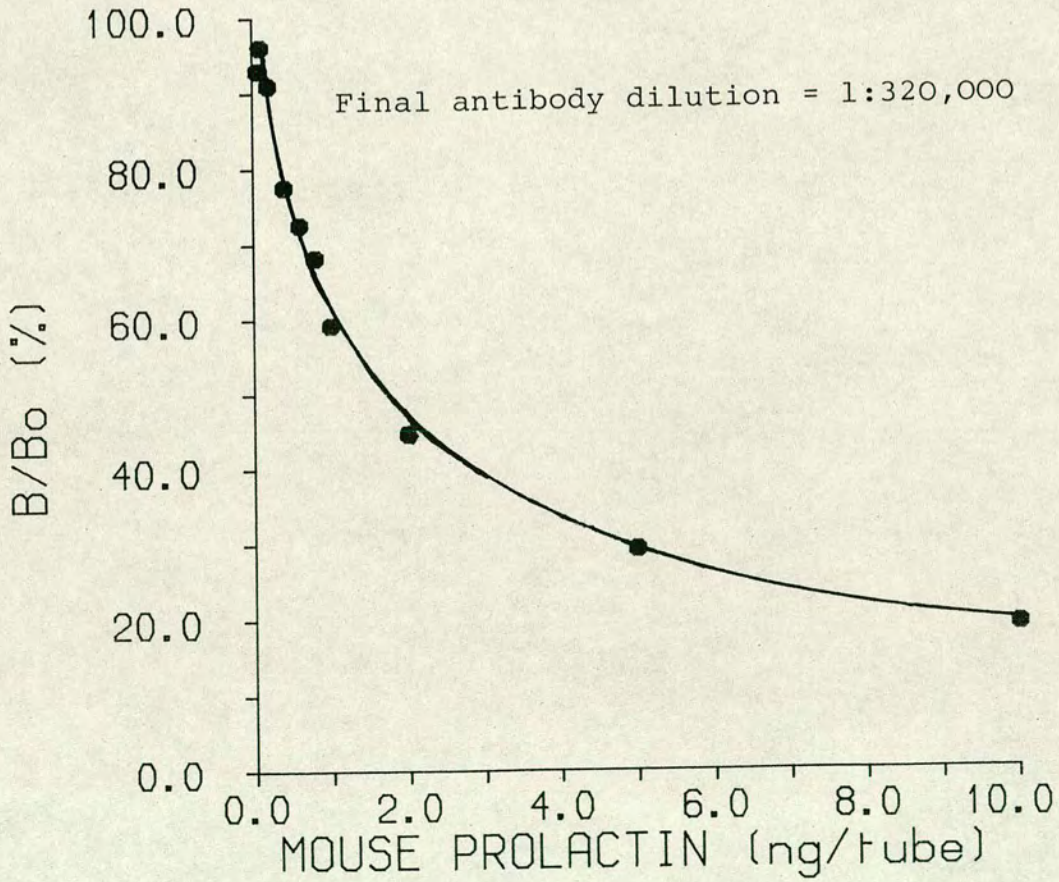


FIGURE 5.2. Standard curve for the radioimmunoassay of mouse prolactin, where bound labelled hormone/amount bound in the absence of free hormone (B/Bo) (%) is plotted against the concentration of standard hormone added (ng/tube). Each point is the mean value of triplicate determinations.

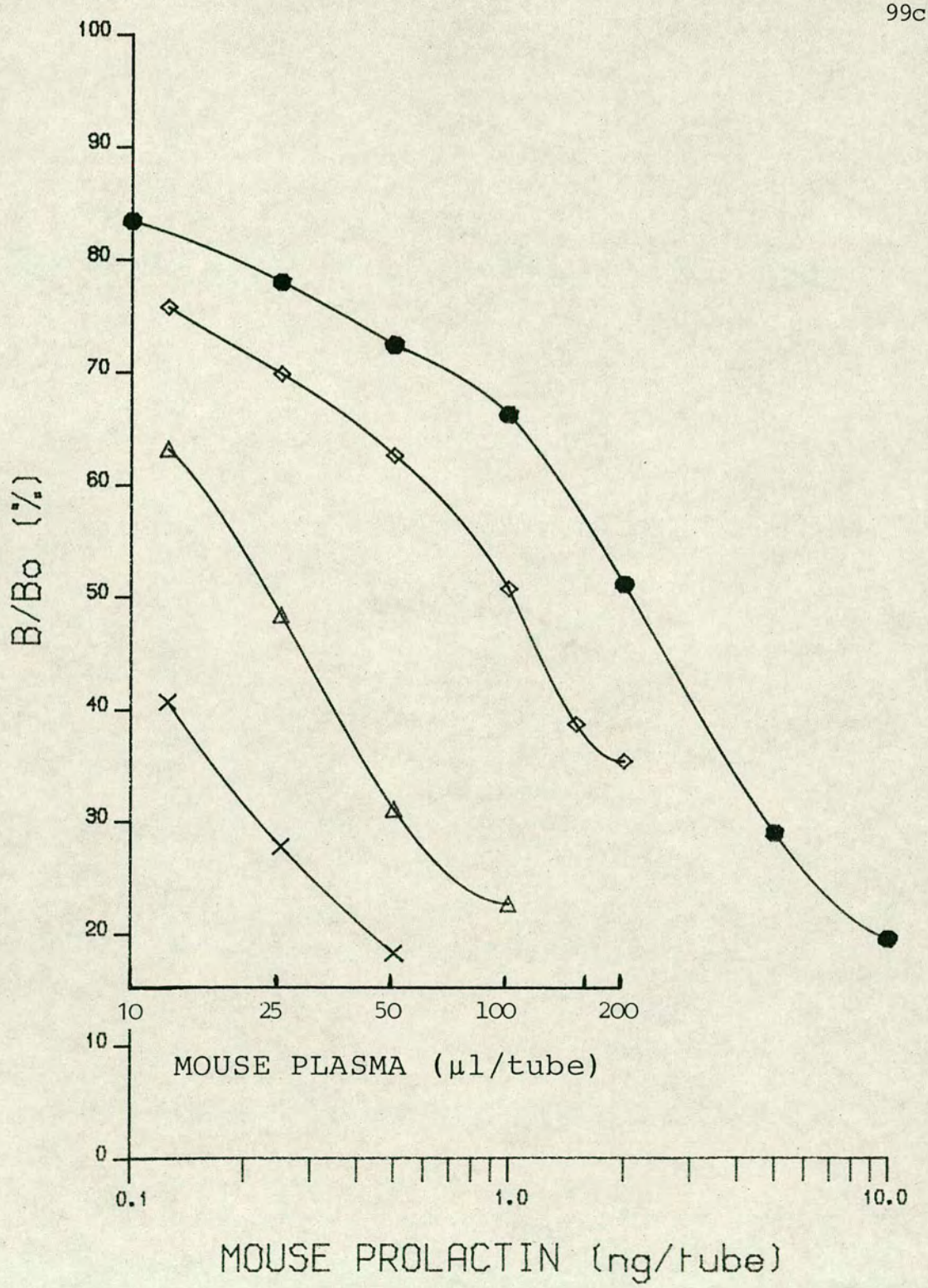


FIGURE 5.3. Dilution-response curves of normal mouse plasma (sampled by decapitation) Δ——Δ, plasma from mice under ether stress X——X and plasma from decapitated mice given bromocryptine injections ◇——◇ in comparison to standard mouse prolactin ●——●.

hormone and the hormones in the plasma samples. It also shows the difference obtained in hormone concentration from the two different methods of blood sampling.

For the test of accuracy of the assay, a linear regression analysis of the results of adding known quantities of prolactin (4, 10, 20 and 40ng/ml) to 25ul of pooled mouse plasma gave the following equation. If y = amount recovered and x = amount added, then $y = 1.41x + 1.91$; the sample regression coefficient, $b = 1.41$, as an estimate of the true regression coefficient, (β) had a confidence interval of $P [1.00 < \beta < 1.82] = 0.975$. In other words, approximately 41 percent more hormone was recovered from the assay on average than was put in.

The interassay coefficient of variation was 16.7% for the high quality control (9 assays) and 11.6% for the low quality control (4 assays). The intra-assay coefficient of variation averaged 6.0%.

5.4. DISCUSSION

The criteria of precision and immunological identity of the assay procedure have been satisfied in general terms. Although in the test of accuracy, more prolactin was recovered quantitatively than added, the discrepancy is not serious and would not bias the relative difference between measurements on unknown samples. Moreover, the ability of the assay procedure to detect differences in prolactin concentration between plasma from mice under ether stress and that from decapitated mice (Figure 5.3) reported in the published literature (e.g. Sinha et al., 1972b) is further demonstration of validity. Absolute concentrations obtained were also similar to those reported by other workers (Sinha et al., 1972a and b).

6. GENERAL DISCUSSION

The overall aim of the studies reported in this thesis has been to obtain a greater understanding of the genetic and physiological relationships between components of growth and reproductive performance in mammals.

The method used has been to investigate the correlated responses in reproductive performance in mice selected for one of three criteria: either appetite, fat percentage or total lean mass.

Laboratory species have been used extensively in pilot studies, for developing a basic knowledge of genetics and physiology relevant to the larger mammalian species. One past example is the finding of a positive genetic correlation between ovulation rate and testis size in the mouse (Land, 1973; Islam, Hill and Land, 1975) which subsequently led to the discovery of a similar relationship in sheep (Land and Carr, 1975; Land, Carr and Lee, 1980). This particular genetic association is being further investigated as a possible selection criterion in commercial sheep breeding.

It is at this level of developing preliminary information, rather than the level of direct extrapolation of results from one mammalian species to another, where laboratory animals can contribute to knowledge most beneficially. Indeed, several workers have cautioned against direct extrapolation of results (Robertson, 1959; Chapman, 1961; Roberts, 1965; Falconer, 1967b). The implications of the work with mice reported in this thesis to other mammalian species is given later in this discussion section.

The mice used in all the studies reported in this thesis came from the selection experiment first described by Sharp et al. (1984). An account of the reproductive performance of the lines

during the first 10 generations of selection has been given in Section 2 and Appendix 1. Another 9 generations of selection have elapsed since those results were obtained, and the additional information is presented below. In Figure 6.1, correlated responses of litter size are shown for the mean over replicates. As in Section 2, litter size is plotted against the generation number of the progeny and represents the reproductive performance of the previous generation of parents. In Figures 6.2 and 6.3, the divergence for litter size between high and low lines is plotted against that for the direct character in the A and P lines, respectively.

The large correlated responses in litter size obtained in the same direction as selection for appetite were quite unexpected. It is difficult to conclude whether they have decreased in later generations relative to direct responses, especially as the between-generation variation in response in both direct and correlated traits appears to have increased following the halving of population sizes of the lines after generation 8. On balance however, the evidence suggests a decline. No obvious differences in litter size are apparent among the F lines after 19 generations of selection for and against high fat percentage.

The observation of correlated responses in litter size in the same direction as selection for total lean mass was expected, given the similarity of the selection criterion to body weight itself, as correlated responses in reproductive performance have been widely reported when body weight or gain have been directly selected (See Tables 1.1 and 1.2). Since generation 10, correlated responses in litter size in the P lines have continued, and largely follow responses in the direct character under selection.

In Section 2, it was shown that ovulation rate had changed in the A and P lines to bring about the correlated responses in litter size, with only minor alterations in pre-natal survival. No

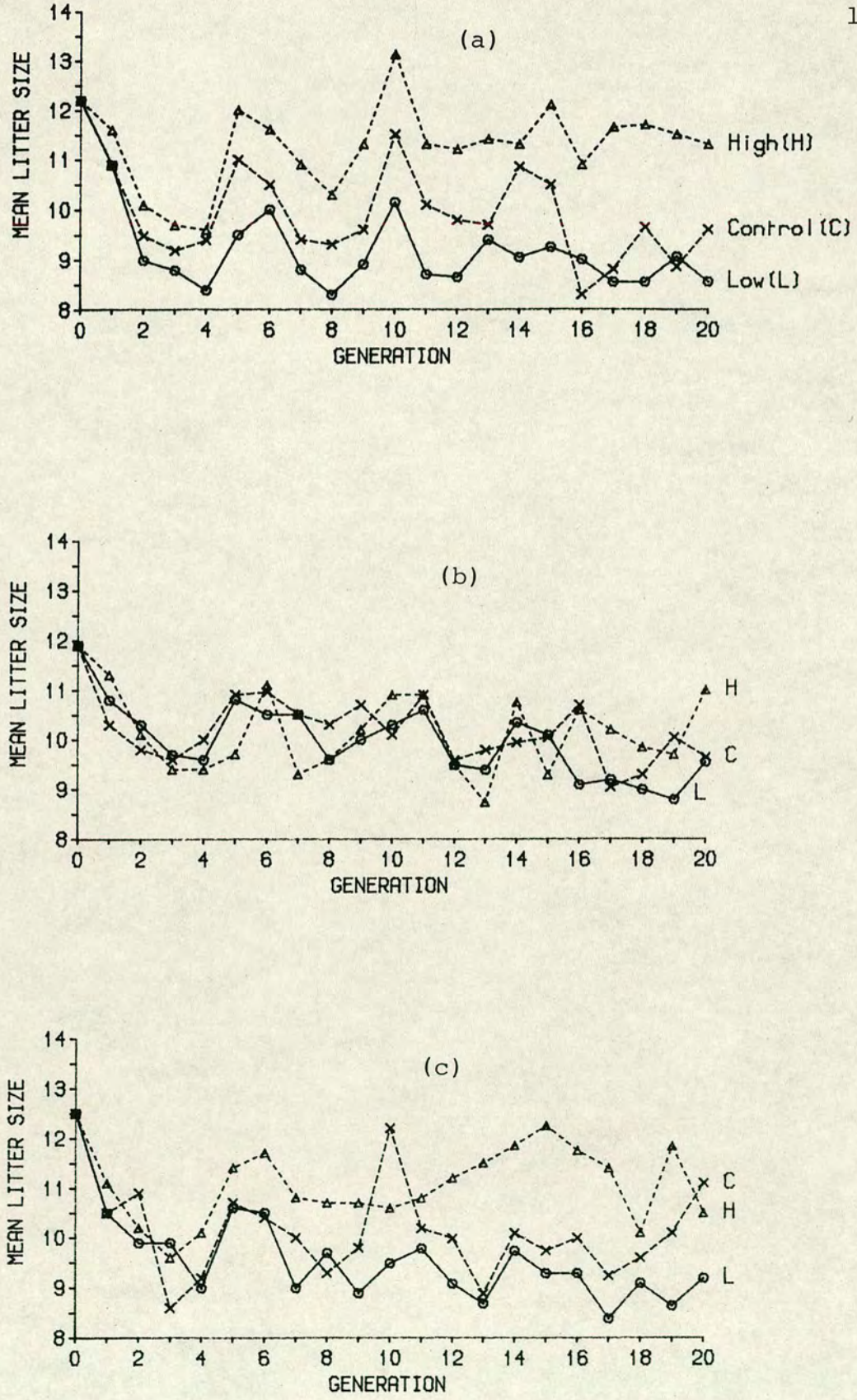


FIGURE 6.1. Litter size for the mean of all replicates for (a) the appetite lines, (b) the fat lines and (c) the lean mass lines. Generation numbers are those of the progeny to correspond with those in Section 2.

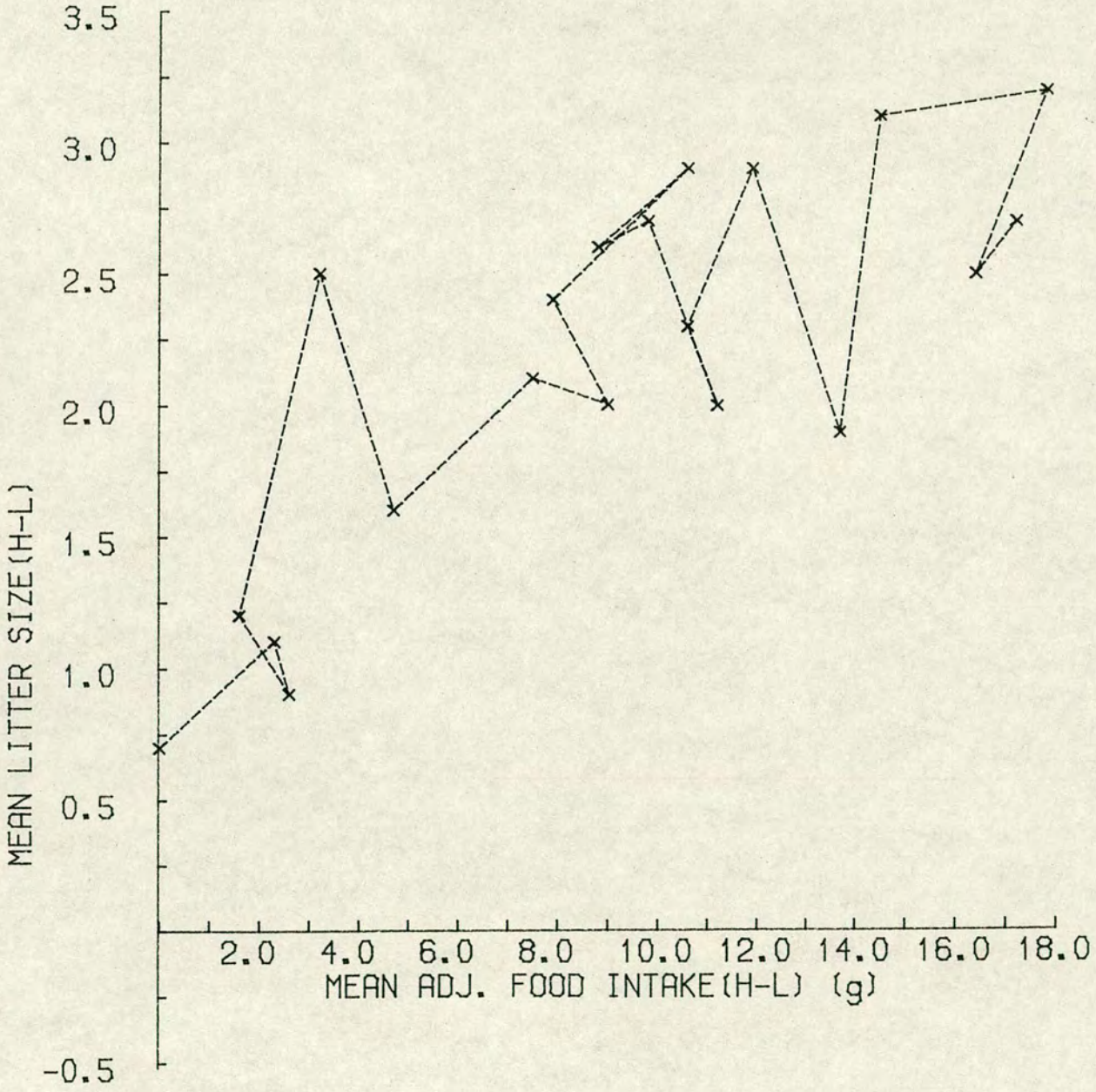


FIGURE 6.2. A LINES: High-low divergence of litter size plotted against high-low divergence of adjusted food intake, for the mean of all replicates.

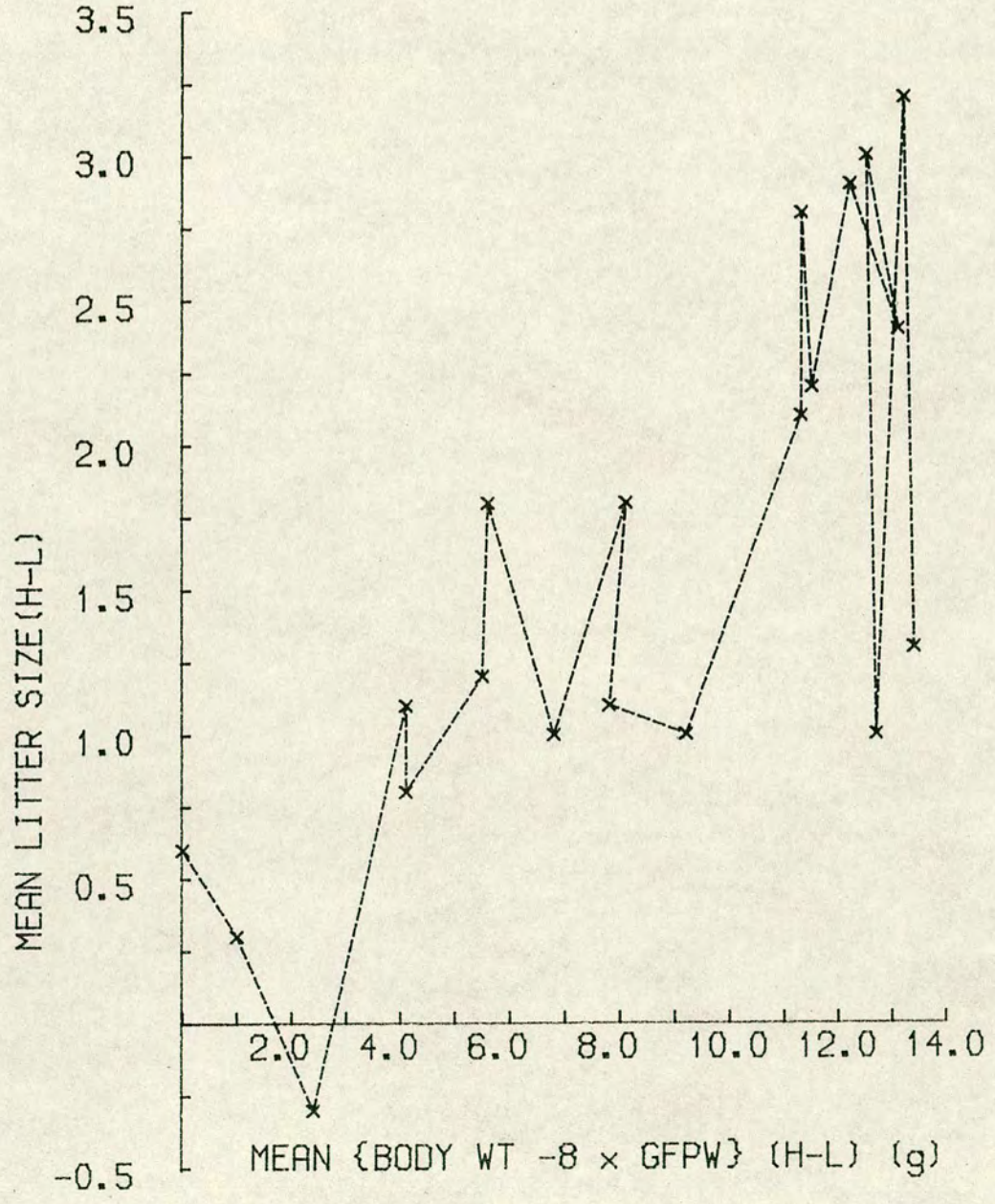


FIGURE 6.3. P LINES: High-low divergence of litter size plotted against high-low divergence of body weight - 8 x gonadal fat pad weight, for the mean of all replicates.

changes were found in ovulation rate in the F lines.

The observation that associated changes in body weight in the A lines could only explain some of the changes in ovulation rate and litter size prompted several hypotheses to be constructed, two of which have been tested in part by further studies and experimentation. These hypotheses were listed in Section 2 and will now be discussed in the order that they were given.

(i) A major gene or genes with large effects on ovulation rate could have been present in the base population.

The reason for offering this as a possibility is that large correlated responses in litter size in the A lines were established rapidly during the early generations of the selection experiment, before there had been time for substantial direct responses to accumulate.

Before specifically discussing major genes, one must ask firstly whether the correlated responses in litter size during the early generations of selection, as governed by selection intensity, genetic correlations and heritabilities, are compatible with the direct responses which could be achieved by selecting on litter size itself.

The direct response to selection (R_x) per generation is given by the formula:

$$R_x = 1/2(i_m + i_f)h^2_x \sigma_{p_x} \qquad \text{(Falconer, 1981)}$$

where i_m and i_f = standardized selection differentials of males and females, respectively, for the trait under selection

h^2_x = heritability of the trait under selection

σ_{p_x} = phenotypic standard deviation of the trait under selection.

Using the following population parameters for litter size of:

$i_m = 0$ (selection on females only)

$i_f = 1.1$

$h^2_x = 0.11$ (Falconer, 1981)

σ_{p_x} = 2.6 young born (From base population of the G-strain, S. King, personal communication).

Then $R_x = 0.16$ young born per generation, or 1.6 young born after 10 generations of selection, where selection has been in one direction and in one sex only.

For a correlated response in Trait X (CR_x) to selection on Trait Y, the following formula gives the predicted response per generation:

$$CR_x = R_A h_x h_y 1/2(i_m + i_f)\sigma_{p_x} \quad \text{(Falconer, 1981)}$$

where r_A = genetic correlation between Trait X and Trait Y

h^2_x = heritability of Trait X, the correlated trait

h^2_y = heritability of Trait Y, the selected trait

i_m and i_f = standardized selection differentials of males and females, respectively for Trait Y, the selected trait.

σ_{p_x} = phenotypic standard deviation of Trait X, the correlated trait.

Re-writing, one can estimate what the genetic correlation would have

to be to explain the correlated responses in litter size to selection in the A lines.

$$r_A = CR_x / h_x h_y 1/2(i_m + i_f)\sigma_{p_x}$$

Using the following population parameters

$i_m = i_f = 1.1$ (selection in both sexes, proportion selected is 1 in 3)

$h^2_y = 0.14$ (Estimated from G strain, Sharp et al., 1984)

$\sigma_{p_x} = 2.6$ (as before)

$h^2_x = 0.11$ (as before)

and $Cr_x = 0.13$ (1/20 x high minus low difference in litter size at Generation 10 of the A lines)

Then $r_A = +0.37$. Phenotypic selection will tend to inflate the differences between the high and low A lines, as the females on which litter size has been measured are themselves selected. At Generation 0, the difference between the high and low A lines is a measure of phenotypic selection only, and the first point on the graph in Figure 6.2 indicates a difference of 0.7 young born between the high and low lines due to this source alone. Therefore, the estimate of r_A may also be inflated. A genetic correlation of +0.37 between adjusted food intake and litter size is not large, and might be explained by the presence of pleiotropic genes with small effects on each of the traits. In addition, the actual correlated response in litter size obtained by selection on adjusted food intake is only 40 percent of those predicted from direct selection on reproductive performance, remembering that with direct selection, only females are selected. This suggests it is unnecessary to invoke a hypothesis involving a gene or genes with

major effects on ovulation rate to explain the results.

The variance of litter size within lines selected for appetite has not shown any decline in any of the three replicates, when it is observed as late as Generation 20, as would be expected if a major gene had become fixed in the lines during the early generations of selection. However, a large variance relative to other lines was noticed in the initial study for ovulation rate in replicate 1 of the high line (see Section 2), although this was not observed in the study reported in Section 3, nor was it consistent for litter size over many generations. If a major gene had been present in the base population, then it should have also been present in the F and the P lines. However, nothing in an examination of the variances in litter size (or ovulation rate when studied) of each line would indicate this to be so.

Taken as a whole, the evidence does not suggest that a major gene or genes with large effects on ovulation rate was present in the base population. Furthermore, the size of the correlated response in litter size obtained in the A lines is within the realms of possible outcomes of the selection applied, through the accumulation of genes of small effect. For these reasons, no active research was undertaken to test for the presence of a gene or genes with major effects on ovulation rate.

(2) The high A line mice may ovulate more eggs in response to the dynamic effect of consuming relatively large amounts of food ('flushing').

The concept of 'flushing' comes from observations that fodder or supplements of high protein and energy content given to ewes just prior to and during mating often lead to increases in the number of lambs born per ewe. Coop (1966) argues that there are two components of the flushing response: a static effect, which is associated with body weight of the ewes at mating, and a dynamic

effect, associated with the process of change in body weight or condition immediately prior to and during mating. This dynamic effect may not necessarily be an ovarian response, and may be mediated through changes in embryo survival (Cumming, 1972). More recently, however, a small dynamic effect of flushing on ovulation rate in ewes has been demonstrated using high protein and energy supplements such as lupin grain and field peas (Davis et al., 1981). For more detailed discussions of sheep experiments relating ovulation rate with nutrition and body weight, see Morley et al. (1978) and Smith (1985).

In the studies reported in this thesis, the static effect of flushing can be investigated by using body weight of the female mice at mating as a covariate in the analysis of the data. In Sections 2 and 3 where this has been performed, the differences between the high and low A lines after fitting regressions were +2.1 and +1.8 corpora lutea compared to unregressed values of +3.8 and +4.1, respectively, indicating a large ovarian response not associated with body size. In relation to values in control lines, the differences in ovulation rate between the high and low A lines after fitting regressions are 17.5% and 10.8% for the studies at first litter and fourth litter, respectively.

It seems unlikely that a dynamic effect of flushing could adequately explain these large residual differences in ovulation rate, for two reasons. Firstly, as there have been correlated changes in basal metabolic requirements in the mice selected for appetite (S. Bishop, personal communication), the amount of food available above maintenance requirements may not be greatly different between the high, low and control lines. If flushing, as defined, is not mediated by alterations in metabolic rate, the minor differences in food available above maintenance requirements among the A lines would probably not be enough to influence ovulation through a flushing mechanism. If, on the other hand, alterations in metabolic rate are involved in flushing responses, then it could be

argued that it is metabolic rate which is the causal factor in altering ovulation rate; in this case, flushing and metabolic rate would be inextricably linked from an experimental point of view. Secondly, dynamic flushing responses in mice of the A lines are likely to be small if it is assumed that the phenomenon is similar in magnitude to that reported in sheep.

The largest dynamic effects of flushing reported in the literature occurred when 'flushed' but not control ewes had access to diets of particularly high protein content (Smith, 1985); after accounting for responses associated with changes in body weight, these diets have given dynamic flushing increases of 15 to 20 percent in ovulation rate (e.g. Davis et al., 1981). It must be remembered that mice in the selection lines all had access to the same diet and their only means of altering protein and energy intake was by changing the quantity of food they consumed. Relative to the scale of differences in protein and energy intake between treatment groups in the sheep experiments cited above, the difference in the amount of food consumed in the high and low A lines of mice is small. Therefore flushing responses, if they exist in the mice, are also likely to be small.

The decision not to research flushing as a possible explanation for the differences in reproductive performance in the A lines was not taken solely on the reasoning discussed above, but also on the knowledge that a suitable experimental design would have been difficult to construct to test the hypothesis. It is therefore useful at this point to digress from the discussion of results and consider in more detail the reasons for not choosing to perform certain kinds of experiments.

'Cafeteria' feeding has been reported to encourage young male rats (Rothwell and Stock, 1979) and mice (Trayhurn et al., 1982) to markedly increase their voluntary energy intake. This technique was considered as a means of flushing but was not pursued since

other people had obtained disappointing results. Mice fed a cafeteria diet at the Genetics Department, University of Edinburgh only had slightly higher energy intakes than contemporary mice fed on a normal stock diet (G. Sharp, personal communication). In addition, to demonstrate flushing responses, female mice would need to be used, and it may be more difficult to encourage them to overeat than male mice. Experimental evidence suggests that female rats show less dependence upon short-term factors (like the stimulus of a cafeteria diet) for controlling feeding behavior than do male rats (Nance, Gorski and Panksepp, 1976). The technique would have also required a large amount of technical work to accurately measure energy and protein intakes (Hervey and Tobin, 1981; Trayburn et al., 1982). However, cafeteria feeding in mice may yet prove to be useful for the study of growth and metabolism in general; further trials are being conducted at Edinburgh University to investigate the effectiveness of the technique.

At first impression, an approach to test the phenotypic relationship between food intake and ovulation rate would be to restrict the amount of food consumed in mice from the lines selected for high food intake and measure the ovarian response in egg number. For instance, Meyer and Bradford (1974) report that feed restriction in mice did reduce ovulation rate in two populations, one previously selected for increased ovulation rate and the other an unselected line. They contrasted this finding to underfeeding a line previously selected for rapid growth, where the decline in ovulation rate was less marked. On closer examination of the literature however, the usefulness of feed restriction experiments for testing for the presence of flushing responses would appear to be limited, for several reasons. Firstly, both acute and chronic underfeeding inhibit reproduction in rodents by interfering with normal secretion of gonadotrophic hormones (McClure, 1967; Howland, 1976). One might argue that the correlated responses in ovulation rate in the A lines are partly mediated by physiological mechanisms analogous to those which mediate the effects of

underfeeding. Quite apart from the fact that one would not be in a position to know whether this was true or false if such experiments were performed, the literature suggests the presence of at least one mechanism which would complicate interpretation.

When female rats are fasted for 72 hours, oestrogen-stimulated LH secretion is inhibited (Dyer and McClure, 1981). This effect apparently involves opiate receptors and is not a direct consequence of reduced blood glucose concentration (Corbet, Dyer and Mansfield, 1982). Although these studies relate to acute underfeeding, they serve as a warning of what could happen under less severe feed restriction. Referring back to the chronic underfeeding study of Meyer and Bradford (1974), there was a delay in mating, with an increased occurrence of cumulus breakdown at autopsy, both as a result of treatment. These are suggestive of an altered physiological environment to that expected in the case of the selection experiment, where feeding was unrestricted. It is worth noting here that no delay or advancement in mating was noticeable in the high or low A lines (see Section 3).

A short period of unrestricted feeding after feed restriction and immediately prior to ovulation may overcome the inhibition of release of gonadotrophins; Cooper, Haynes and Lamming (1970) found that this regimen of nutrition in female rats enabled the animals to ovulate as many eggs as those on ad-libitum feeding. Even with this additional procedure, interpreting the results would still be a big problem, as the treatment may still be an unsatisfactory model of how flushing responses are mediated.

A change in the general metabolism of mice which are underfed is a possibility, and this might also complicate the attempt to draw a parallel between the experimental animal and flushing responses in the A line mice which are fed ad-libitum.

To return to the discussion of results:

(3) Selection for high appetite may have produced mice which reach their "peak" of reproductive potential earlier in life than lines selected for body weight, or components thereof.

This hypothesis was tested in the study reported in Section 3. No alteration in the timing of the peak of reproductive output could be found among the high, control and low A lines. In addition, the pattern of differences of litter size in the A lines over the 4 parities was similar to that in the P lines, i.e. those lines selected for a trait closely related to body weight. The hypothesis therefore was found to be false.

(4) There may be some pleiotropy between genes controlling food intake and metabolic rate and those controlling ovulation rate.

The first evidence of differences in metabolic rate came from feed restriction studies, where the high A line mice required 10 percent more food for maintenance than those of the low line, the difference being expressed as a percentage of the control line mean (M. Nielsen, unpublished). Since then, two methods have been used to estimate metabolic rate, the first by inference from food intake and growth rate data, and the second, a more direct measure, by recording basal heat production in fasted mice. The high A lines had a 13 percent higher basal metabolic rate than those of the low A lines, when the difference is expressed as a percentage of the control line mean. This figure is based on several recordings taken during the active growth phase of the mice, i.e. up to 10 weeks of age (S. Bishop, personal communication).

There is no direct evidence in the literature of genetic relationships between metabolic rate and ovulation rate. McNab (1980) suggests that there is an association between basal metabolism and reproductive rate after studying wild populations of various mammalian species. His ideas are based on the importance

of basal metabolism for population control and fitness in a natural selection context.

If it is assumed that the metabolic rate changes in the A lines are the primary cause of the changes in ovulation rate and litter size, the suggested diminishing rate of correlated response in litter size in the later generations of the selection experiment might be explained as follows. The selection criterion used in the A lines includes a correction for body weight. This correction makes the criterion sensitive in its efficiency in changing metabolic rate to small changes in the variance and covariance relationships of body weight with food intake (S. Bishop, personal communication). After a number of generations of selection, a change in these relationships may have resulted in a diminishing of the response in metabolic rate and therefore ovulation rate.

Alternatively, the first genes that might have become fixed in the direction of selection for appetite could have been those with the largest effects on ovulation rate and litter size. After fixation of these genes, the selection process would then be acting only on genes with smaller effects on reproductive performance, and as a result, the correlated responses in ovulation rate and litter size to selection on appetite would reduce accordingly.

The experiment reported in Section 4 attempted to relate the hormone prolactin to the relationship observed between appetite and ovulation rate. This was undertaken to develop knowledge of the covariation between the traits. Because of prolactin's apparent lack of involvement in the determination of ovulation rate, I concluded that it was not involved in mediating a relationship between this trait and appetite in the mouse.

Another approach would have been to investigate the physiological mechanisms involved in controlling variation in either the correlated trait, ovulation rate, or the direct trait,

appetite, as separate issues. For instance, the control of ovulation rate could have been investigated by contrasting the high A line with the low A line for gonadotrophin activity and ovarian responsiveness to their action. Although development of this knowledge may have been useful in itself, it would not have directly addressed the question as to why lines of mice selected for appetite respond with correlated responses in ovulation rate and litter size, so it was not pursued.

As mentioned in Section 1.3 of the literature review, it is possible that several hormones are involved in mediating relationships between growth and reproductive performance in mammals. Similarly appetite, as a component of the growth process, may also mediate its relationships with ovulation rate through several or many hormones. The neurotransmitters of the central nervous system should not be overlooked as potential factors in these associations either. The changes in basal metabolic rate in the A lines may be linked with these hormonal and neuro-hormonal factors. The confirmation and testing therefore of the causal relationships between metabolic rate and ovulation rate in future work may help unravel the physiological mechanisms involved.

But how is this to be done? Considering the many factors which are probably involved in the control of rate of metabolism, the task is not likely to be an easy one, although more genetic material will soon be at hand to help. Lines of mice at the Genetics Department, Edinburgh University, are to be selected in the future on food intake between 8 to 10 weeks of age, with a phenotypic correction for body weight. This selection criterion is more related to basal metabolic rate than that used in the A lines, since the food intake measurements are to be taken not at a time when the mice are in the accelerating growth phase (early post-weaning), but later on, when their growth rate is much slower.

These new selection lines will either confirm or refute the

view that there is a general relationship in mice between appetite or metabolic rate on the one hand and ovulation rate and litter size on the other; secondly, they will provide information on correlated responses in reproductive performance to selection on food intake which are not confounded by substantial changes in body weight and gain (as were the A lines) and thirdly, they will help address the question as to whether the litter size alterations in the A lines were obtained because selection was based around the time of puberty, since the criterion to be used will measure mice well after they become sexually mature.

Experimental alterations of metabolic rate, by the application of drugs, exogenous hormones or antibodies to hormones could be fruitful lines of research to see what the physiological relations are between rate of metabolism and ovulation rate. The use of repeated measurements of several hormones is likely to give a more accurate view of the endocrinological situation in experimental animals that single point measurements, as repeatability in time of hormone concentrations is low, especially for hormones secreted from the pituitary gland (Osmond, 1979). The requirement for repeated blood samples would make it difficult to perform this type of work in the mouse. Consideration should be given to conducting this research in a larger mammalian species, after establishing that the genetic relationship exists.

The results of selection for lean mass and percentage fat suggest that in the mouse, the overriding factor genetically associated with ovulation rate and litter size is lean mass. In the F lines, where there have been no correlated responses in ovulation rate and litter size, no changes have occurred in lean mass either. However, there have been large changes in body composition, which are reflected in alterations in body weight. This situation can be contrasted with that in the P lines, where large correlated responses have been recorded in ovulation rate and litter size along with substantial alterations in lean mass, but little, if any

change has been recorded in body composition (Sharp et al., 1984; S. Bishop, personal communication).

The finding of genetic covariation between ovulation rate, litter size and appetite in mice adds another dimension to the understanding of growth and reproductive performance relationships. A general conclusion is that the genetic determination of reproductive rate in the mouse, as measured by ovulation and litter size, is influenced among other factors by the lean mass of the animal and by its appetite in relation to that lean mass.

The study reported in Section 3 revealed that the high fat percentage lines do not have lower fertility or litter size than the control lines when comparisons are made over four parities. The lack of differences in fertility was contrary to expectation, as overfatness has been partly blamed for infertility in lines of mice selected for high body weight or gain (Roberts, 1965, 1979). In fact, when fertility, litter size and survival of the mice were expressed together as the one trait in Section 3, the high fat lines had slightly higher performance than the controls, which in turn, were higher than the low F lines.

In concluding that fatness per se is not genetically associated with female fertility in mice, it is suggested that infertility in lines selected for high body weight or gain by earlier workers (see Section 1) may well have arisen for other reasons. Premature ageing of the uterus and inbreeding effects might be important contributing factors in the relatively poor 'lifetime' performance of these lines when they are studied under systems of continuous breeding. Typically, first litter size has increased relative to that of control mice when researchers have selected for increased body weight or gain (see Section 1). Under continuous breeding, the high initial production of the high weight or gain lines has not been maintained subsequently as -

- litter size at later parities has declined more rapidly than in control lines

- either the female or the male or both members of each breeding pair became sterile.

Wallinga and Bakker (1978) report a similar situation with continuous breeding in a comparison between a line selected for high first litter size and an unselected control line. However, under an interval breeding scheme (i.e. male sires are removed from mating cages before the females give birth, and not replaced until the litters are weaned) they found that mice of the selected line gave birth to a larger total number of young during a period of 228 days than those of the control line. This difference in results between the two methods of breeding suggests that continuous breeding, in combination with the large initial litter size in lines selected for increased body weight or gain (or in Wallinga and Bakker's high litter size line), may have created the conditions of overloading in the uteri of female mice discussed by Finn (1963). He concluded that the decline in litter size under conditions of overloading is caused by an earlier occurrence of ageing effects; normally, chronological ageing effects cause a decline in embryo survival after implantation.

When the effects of inbreeding and heterosis on reproductive performance in mice have been studied, emphasis has often been placed on alterations in litter size and fertility at first litter (Bowman and Falconer, 1960; Falconer and Roberts, 1960; Roberts, 1960; McCarthy, 1965 and review by Eisen, 1974). In general, first litter size declines by about half a mouse per 10% increase in inbreeding coefficient and is restored to approximately its original level on crossing (Roberts, 1981).

Deliberate inbreeding also results in the extinction of most lines through infertility and inviability if the inbreeding

coefficient rises much above 50% (Roberts, 1960), although a small number of lines have managed to survive even higher levels (Bowman and Falconer, 1960; Falconer, 1971). When studying lifetime or long-term reproductive rate however, it would be more useful to know what effects inbreeding have on these traits rather than just on litter size and fertility at first parity.

It is therefore of interest that Nagai, McAllister and Chesnais (1984) have reported large amounts of heterosis in 'lifetime' production traits in mice. As part of their study, Nagai and co-workers crossed two distinct lines, both of which had previously been selected for increased 6 week weight. When F1 males and females from these crosses were continuously bred in pairs for 155 days, the total accumulated number of young born was 46% more than the average performance of the straightbred lines. The sum of male and female components of heterosis for the number of parturitions per female, estimated from the same data set was +26%, in itself a relatively large figure. Furthermore, pairing of F1 males and females from crosses of two unselected populations, which were contempories of the body weight lines of Nagai et al. (1984), showed smaller amounts of heterosis for total young born and number of parturitions per female (these traits were defined above); the figures were +31% and +15%, respectively. This suggests that selection for body weight may have heightened the effect of inbreeding on lifetime reproductive traits. Nagai et al. (1984) report an inbreeding coefficient of 13% for their unselected control populations of mice; in the long-term reproduction studies of Roberts (1961) and von Butler et al. (1984), the inbreeding coefficients of the lines are likely to have been higher, around 15-20%, although the exact figures are unavailable.

The depression in first litter size from the above levels of inbreeding could theoretically amount to 0.65 to 1.0 young per female, using estimates from the literature. Assuming no overdominance, F1 crosses of these lines should show between +6.5 to

+10% heterosis for first litter size when they are themselves mated together (a mean litter size of 10 is assumed). It therefore seems likely that inbreeding can influence 'lifetime' reproductive traits more than its apparent effect on first litter size. These suggestions are obviously based on limited evidence; more research is needed to discover what the actual reasons are for the decline in 'lifetime' reproductive performance in lines selected for increased body weight or gain. Such research may very well lead to a new interpretation of old findings.

Implications for other mammalian species

The existence of a general relationship between growth and reproductive performance within mammalian species, consistent with that in mice (see Section 1), supports the idea that results of studies reported in this thesis are relevant to other mammalia. Selection for lean growth, which is closely aligned to the criterion used in the P lines, is often used in the selective breeding of large animals for meat production. From the point of view of reproductive performance, the mouse results suggest that an increase in the mature weight and/or the growth rate of animals brought about by selective breeding is likely to increase rather than decrease litter size. This agrees in general with the evidence from the literature on sheep and pigs (see Section 1). These improvements in litter size will most likely be reflected in improvements in the number of offspring produced by each female over her 'productive' life (approximately 4 parities), as the study in Section 3 demonstrates this in mice.

Results from the lines selected to alter body composition (the F lines) are particularly relevant to the commercial breeding of pigs, where a reduction in backfat thickness and an increased growth rate are often joint selection objectives. Whether the architecture of genetic control between fatness and reproductive performance is similar in pigs and mice is unclear, but the literature certainly

does not suggest the existence of strong correlations between the traits in the former species. It should be remembered however that the evidence is mainly from European breeds. The lack of any alteration of ovulation rate, litter size, or fertility in mice of the F lines, despite the large changes in body composition, suggests that present policies in breeding pigs with reduced backfat thickness are unlikely to lead to any reductions in reproductive performance.

The slight possibility of selection against fatness in pigs reducing litter size through a reduction in appetite would probably be more than offset by the common practice of using selection indices with positive weightings on lean growth; selection based on such an index has led to increases in food consumption in an experiment with pigs (Vangen, 1977).

Similarly, the results suggest that fatness per se is not strongly correlated genetically with reproductive performance in sheep and cattle. Any sizeable phenotypic relationships which are observed between overfatness and infertility in these two species are probably a result of environmental correlations between the traits.

In humans, it is obviously difficult to partition genetic and environmental sources of correlation, so the results of selection in laboratory species are relevant in the absence of more specific evidence. As preliminary evidence, the mouse results suggest that infertility and obesity or extreme leanness in women are probably not strongly correlated genetically. Environmental sources of correlation, such as nutritional and behavioral factors may well be more important in determining the existence of phenotypic associations between these traits.

The unexpected finding of a genetic relationship between appetite and litter size in the mouse has raised the possibility

that basal metabolic rate may be involved in the genetic determination of ovulation rate. If this is confirmed, there are no *a priori* reasons for doubting that similar relationships would exist in other mammalia.

The reduction in litter size of mice selected for low food intake serves as a caution against applying selection procedures in commercial animal breeding that lead to reductions in appetite. In pigs, selection programmes giving a relatively high weighting to improved feed efficiency and lean content at the expense of growth rate on *ad libitum* feeding show a correlated decline in voluntary food intake (Mitchell *et al.*, 1982). Similarly, declines have been reported from two experimental index selection lines on *ad libitum* feeding (McPhee, 1981; Ellis *et al.*, 1983). Such selection procedures might lead to a decline in litter size through reductions in mature size and through reductions in the proportion of food eaten relative to the lean mass of the animals.

Direct selection for increased appetite itself in the breeding of commercial animals would probably not be appropriate, as the gains in reproductive performance and growth may be more than offset by the cost of the extra food consumed. Direct measurements of food intake in grazing animals would also be difficult and expensive; reliance on the results of pen feeding might reduce the technical difficulties but may, in addition, decrease the accuracy of selection.

Rather than direct selection based on food intake, exploitation of appetite-litter size relationships in an animal breeding context might be through the recognition of critical factors controlling reproductive performance; this knowledge would aid in the definition of suitable criteria for selection.

In sheep, the rate of change in litter size achieved by direct selection within flocks has been slow (see reviews by Turner, 1969

and Bradford, 1972). Four main factors are responsible for this slow response: firstly, the heritability of litter size is low; secondly, the trait is sex limited, and can only be measured in females; thirdly, the trait can often not be measured until the ewes are two to three years of age and fourthly, the low reproductive rate itself limits the selection pressure which can be applied (Land, 1974).

The ability to select males on criteria genetically correlated to female performance would enhance the selection intensities that could be applied in the breeding of sheep and perhaps livestock in general. This reasoning has been given before by Land (1974). For sheep breeding, Walkley and Smith (1980) have quantified the advantages of using physiological criteria in males to improve reproductive merit in females. It is therefore of interest that the genetic associations apparent between appetite and ovulation rate in female mice appear to have their corollary in males. Evidence is now accumulating that testis size (in relation to body weight) in males of the high and low appetite lines has also changed in the direction of selection (P. Cook, personal communication), although analysis and interpretation of the data is at a preliminary stage. The prospect of finding similar relationships in sheep, if confirmed in mice, would appear to be high, as demonstrated by Land and his co-workers for ovulation rate and testis size relationships (see the start of this Section).

To summarize and conclude, the results of studies with mice reported in this thesis improve the knowledge of relationships between reproductive performance and components of the growth process in most, if not all the mammalia. More specifically, they:

1. add confidence to the view that selection for increased lean growth in commercially bred animals will probably lead to increases rather than decreases in litter size. Provided animals are not kept in agricultural production systems for longer than they are at

present, these increases in litter size may give improvements in total reproductive rate, since fertility is unlikely to be adversely affected by selection.

2. demonstrate that fatness per se in mice is not genetically correlated with litter size and fertility. This confirms the tentative evidence for lack of such relationships in pigs, and suggests that infertility problems in sheep, cattle and humans are probably not strongly correlated genetically with fatness.

3. raise the possibility that reproductive performance in mammals is positively correlated genetically with appetite and basal metabolic rate. If confirmed in the species of commercial interest, an increased understanding of these relationships may help identify suitable selection criteria for improving reproductive merit.

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Effects of selection on growth, body composition and food intake in mice

II. Correlated responses in reproduction

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SUMMARY

Female reproductive performance is reported in mice selected for ten generations for one of three criteria: either appetite (*A*), fat percentage (*F*) or total lean mass (*P*). For each criterion lines were selected for high (*H*) or low (*L*) performance, with contemporary unselected controls (*C*). In the *A* and *P* lines, litter size changed in the direction of the selected criterion, the changes being larger and more rapidly established in the *A* than in the *P* lines. At generation 10, the differences in litter size between high and low lines were 2·6 live young born in the *A* lines, and 1·0 live young born in the *P* lines. The differences in 6-week weight between the high and low lines were 3·5 g in the *A* lines, 6·5 g in the *P* lines. Changes in ovulation rate were the primary reason for changes in litter size, the differences between the high and low lines being 3·8 corpora lutea for the *A* lines, and 3·1 corpora lutea for the *P* lines. Fitting body weight at mating as a covariate within lines in the analysis of ovulation rate and live foetus number removed the differences between the high and low selected *P* lines, but not those in the *A* lines. The high and low selected *A* and *P* lines did not differ in prenatal survival. There were no consistent differences in litter size, ovulation rate or pre-natal survival in the *F* lines.

1. INTRODUCTION

Reproductive performance is important in determining profitability of many animal production systems, so its genetic determination and interrelationships with other major traits, namely growth rate, body composition and food intake are important to the animal breeder.

The mouse has been used extensively as a model to help understand the basic genetic and physiological mechanisms involved in traits of importance in larger mammalian species. Reproductive performance has been investigated in outbred populations of mice either by studying lines selected for litter size, or its components, ovulation rate and embryonic survival, or by studying it as a correlated trait to selection for other traits. In almost all published reports of reproductive performance as a correlated trait in mice, selection has been practised

for body weight or growth rate (for reviews, see Roberts, 1965, 1979, and McCarthy, 1982). In these published studies, litter size has been used as a measure of reproductive performance, and has usually changed in the direction of selection (e.g. MacArthur, 1949; Falconer, 1953; Rahnefeld *et al.* 1966), but not in all cases (Bradford, 1971). Changes in ovulation rate in the same direction as changes in body weight have been shown to be the primary reason for the associated responses in litter size (MacArthur, 1944; Fowler & Edwards, 1960; Land, 1970), although the biological mechanisms involved in these relationships are not understood.

Lines of mice have been selected in our laboratory for one of three criteria, appetite, fat percentage or total lean mass (Sharp, Hill & Robertson, 1984). In this paper the correlated responses in litter size after ten generations of selection are reported. To understand these responses in litter size more fully the major components of litter size, namely ovulation rate and pre-natal survival were investigated.

2. MATERIALS AND METHODS

(i) *Selection lines*

Mice were selected for one of the three criteria: appetite (A) measured as 4- to 6-week food intake, corrected by phenotypic regression for 4-week body weight, fat percentage (F), using the ratio of gonadal fat pad weight (GFPW)) to body weight (BW) in 10-week-old males, and total lean mass (P), using the index $BW-(8\times GFPW)$ in 10-week old males.

For each selection criterion, there were three contemporary lines, one selected for high (H) performance, one for low (L) performance together with an unselected control (C). These lines were replicated three times (replicates 1, 2 and 3) for each of the three selection criteria. Thus, there were 27 lines maintained in all: 3 selection criteria $\times$ 3 replicates $\times$ 3 directions (H, L and C). Sixteen pair matings were made in each line up to generation 8; subsequently 8 pair matings were used. Selection was practised within litters. In the A lines, both sexes were selected. In the F and P lines, females were taken at random.

A full account of the origins of the mice, selection procedures and the responses obtained in growth, food intake and body composition for the first 11 generations, is given by Sharp *et al.* (1984). Each generation, 6-week weights, litter size at birth (number of live young) and those born dead were recorded in all the lines.

Mothers of generations 4 and 10 were given terramycin antibiotic in the water supply for the first week post-partum. This was done to alleviate the effects of an unidentified disease which caused ill-thrift in suckling litters and, in acute cases, death of the mother during the peak of lactation.

(ii) *Analysis of ovulation rate and pre-natal survival*

Mice and management. Random samples of mice not chosen as parents for the selection lines were taken from each of the 27 lines (replicates 2 and 3 from generation 9 and replicate 1 from generation 10) and pair mated to produce mice for measurement in this study. These mice were thus contemporaries of those used for breeding in generations 10 (replicates 2 and 3) and 11 (replicate 1) of the selection lines. In addition, a small number of mice not chosen for matings in generation

10 and 11 of the selection lines were used; in the *A* lines these mice had been measured for the selection criterion. Mothers of generation 10 (Replicate 2 and 3), but not generation 11 (Replicate 1) were given Terramycin antibiotic in their water supply for the first week post-partum. As in the main selection lines, litters were adjusted to between 6 and 12 pups at birth, weaning took place at 21 days of age when the sexes were separated, and weaned mice were held in stock cages (no more than 6 mice in each cage) until mating time.

Females were weighed and mated at 8 weeks of age except in replicate 3 of the *F* lines which were weighed and mated at 7 weeks, by mistake. Two females were mated to each male, except where close inbreeding could be avoided by pair mating or mating three females to each male. Allocation of mates was similar to a scheme designed by Falconer (1973). The set of three lines, *H*, *L* and *C* of each replicate of each selection criterion were contemporaneous, as during the selection experiment.

Dissection technique. Vaginal plugs were used to indicate the day of conception, and females were dissected after 17 days to measure ovulation rate and pre-natal survival. Ovulation rate was estimated by counting the number of corpora lutea on each ovary under a dissection microscope.

This method is liable to underestimate ovulation rate, particularly when the corpora lutea are numerous, because of the difficulty of distinguishing between one large corpus luteum and two adjacent and partially confluent ones. To improve the accuracy of the count, each corpus luteum was dissected out under the binocular microscope with an eye surgeon's scalpel. In 17 out of the 556 pregnant mice studied (3·1 %) there were more implants in one horn of the uterus than corpora lutea counted on the adjacent ovary; in these cases the latter count was adjusted upwards to equal the number of implants. Although migration of embryos from one horn of the uterus to the other (McLaren & Michie, 1954) or polyovular follicles (e.g. Kent, 1960) might account for the discrepancy, we consider an error in counting to be a much more likely cause. As this is revealed only where there is no pre-implantation in one or both horns of the uterus, a count of corpora lutea could underestimate ovulation rate in more than the 3·1 % of cases corrected; but because all lines were counted in the same way, this bias should not seriously affect the conclusions (as argued by Falconer & Roberts, 1960).

The number of live foetuses and post-implantation losses (moles + resorptions + dead foetuses) were also recorded, and percentage survival computed as the ratio of live foetuses to corpora lutea.

Statistical analysis. Body weight, ovulation rate, live foetus number and pre-natal survival were subjected to analyses of variance by least squares. The main model fitted to the data was:

$$Y_{ijklm} = \mu + T_i + D_{ij} + R_{ik} + L_{ijk} + F_{ijkl} + e_{ijklm},$$

where Y_{ijklm} is the observation on the mth individual of the lth full-sib family of the kth replicate of the jth direction of selection and the ith selection criterion. Also: μ is the overall mean; T_i is the effect of the ith selection criterion ($i = 1, 2, 3$ corresponding to *A*, *F* and *P*); D_{ij} is the effect of the jth direction of selection ($j = 1, 2, 3$ corresponding to *H*, *L* and *C*) within the ith selection criterion; R_{ik}

is the effect of the kth replicate ($i = 1, 2, 3$) within the ith selection criterion; L_{ijk} is the effect of the individual line and is used to estimate the effects of drift; F_{ijkl} is the full-sib family effect; and e_{ijklm} is the residual within full-sib family effect.

Directions of selection and replicates were tested against lines, pooled over selection criteria.

In further analyses, terms were also added for linear regression on body weight and/or ovulation rate of the individual mouse.

(iii) *Repeat sampling of Replicate 2 of the A lines*

The mice used in replicate 2 of the *A* lines were thought unrepresentative, as indicated by their body weights (see Results). Therefore, using the same procedures an additional study was conducted on this replicate on progeny of mice not selected for generation 12 in each selection line. The mothers of dissected mice did not receive Terramycin antibiotic.

Both the original and the repeat samples contributed to the results analysed, with the repeat sample included as an extra replicate. The bias created by this procedure was corrected for by reducing the sums of squares for the main effect of replication and the interaction of replication with direction of selection (called 'lines').

3. RESULTS

(i) *Correlated responses in litter size*

The mean litter sizes each generation from 0 to 10 are shown in Figs. 1–3, for each replicate and for the mean over replicates. To conform with the graphs of Sharp *et al.* (1984), litter size is plotted against the generation number of the *progeny*, and represents the reproductive performance of the *previous* generation of parents.

There was a rapid initial decrease in litter size in all lines. A decrease could be expected between generations -1 and 0 (0 and 1 of the progeny, Figs. 1–3), as those of generation -1 were a three-way cross (Sharp *et al.* 1984) with maximum heterosis for litter size. Subsequently, assuming unrelated founder animals, the range in inbreeding coefficients for lines at generation 10 was 5·7 %–9·0 %, with a mean of 6·8 % and no consistent difference in breeding between selection criteria, directions of selection or replicates. As Falconer's (1973) scheme for minimal inbreeding was used in the selection lines, no inbreeding accrued until generation 4, so inbreeding can not explain the initial decline in reproductive performance. A more likely source of the decline in litter size in the early generations of selection could have been a general decline in the health of the mice, as evidenced by very small young at weaning time and, in acute cases, by deaths of suckling females. Terramycin antibiotic was administered to the mothers of generation 4 and 10, and the offspring of generation 4 had, on average, larger litters than the previous generations.

Large and consistent differences in litter size between the high, low and control *A* lines were rapidly established. There were smaller but consistent differences between the high and low *P* lines, but no consistent differences among the *F* lines.

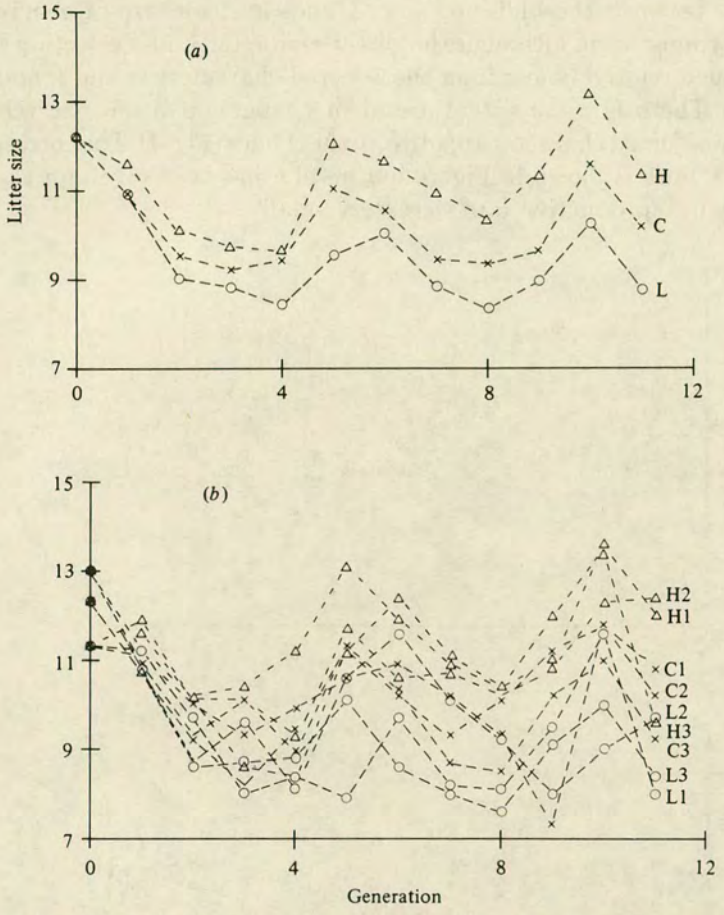

Fig. 1. *A* (appetite) lines: litter size for (*a*) mean of all replicates, (*b*) individual replicates. Generation numbers are those of the progeny to correspond with those of Sharp *et al.* (1984).

Table 1. *Mean of selected character and female 6-week weight in each set of lines at generation 10 (replicates pooled)*

	Direction of selection			
Selection criterion	High	Control	Low	High –Low
A (adjusted food intake) (g)*	66·3	63·3	57·5	+ 8·8
6 weeks wt (g)	26·0	23·4	22·5	+ 3·5
F (gonadal fat pad wt/body wt)† (mg/g)	20·5	14·0	8·7	+11·8
6 weeks wt (g)	23·6	23·7	22·2	+ 1·4
P (body wt − 8 × gonadal fat pad wt)† (g)	34·8	29·0	25·6	+ 9·2
6 weeks wt (g)	26·7	22·8	20·2	+ 6·5

* Adjusted food intake (g): FI + 1·65 (16·1 − *w*) for females, FI + 2·21 (17·8 − *w*) for males, where FI = 4–6 weeks food intake (g), *w* = 4 weeks wt (g).

† Body weight and gonadal fat pad weights measured in males at 10 weeks of age.

The difference between the high and low *A* lines in litter size of generation 10 females is 2·6 young born, although the direct character under selection in the *A* lines has changed relatively less than the selected characters in the *P* and *F* lines (see Table 1). There is a consistent trend of changes in litter size relative to responses in the selected character appetite, in the *A* lines (Fig. 4). The corresponding graph for the *P* lines is shown in Fig. 5, but no plot has been given for the *F* lines where changes in reproductive rate were very small.

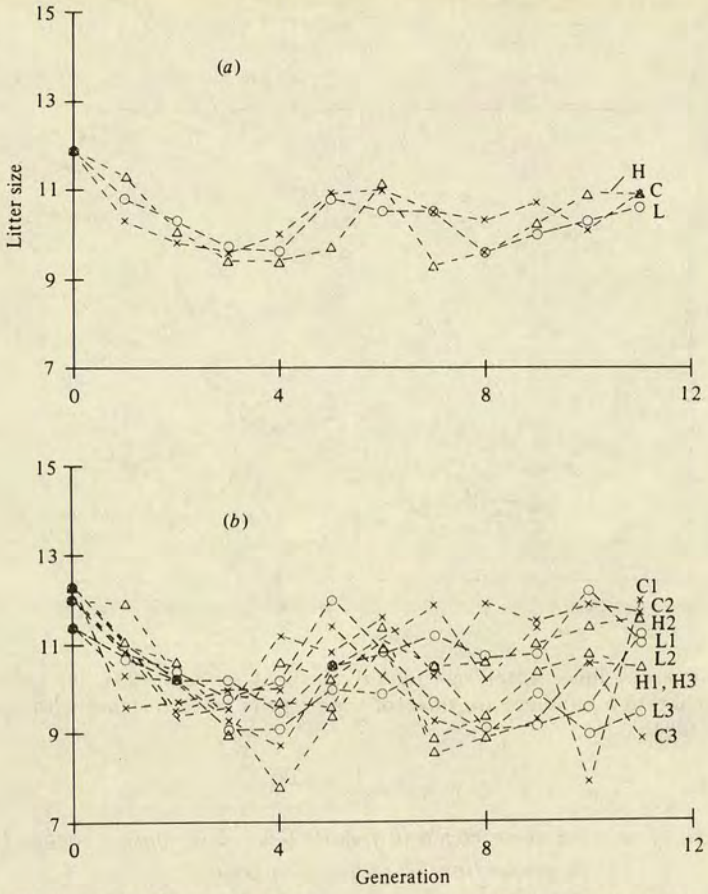

Fig. 2. *F* (fat) lines: litter size for (*a*) mean of all replicates, (*b*) individual replicates, as Fig. 1.

At generation 10, the mean difference in 6-week weight of females between high and low selected lines was 3·5 g for the *A* and 6·5 g for the *P* lines (Table 1). However, despite these larger differences in female body weights in the *P* compared to the *A* lines, the subsequent difference in litter size between the high and low *P* lines was only 1·0 young born.

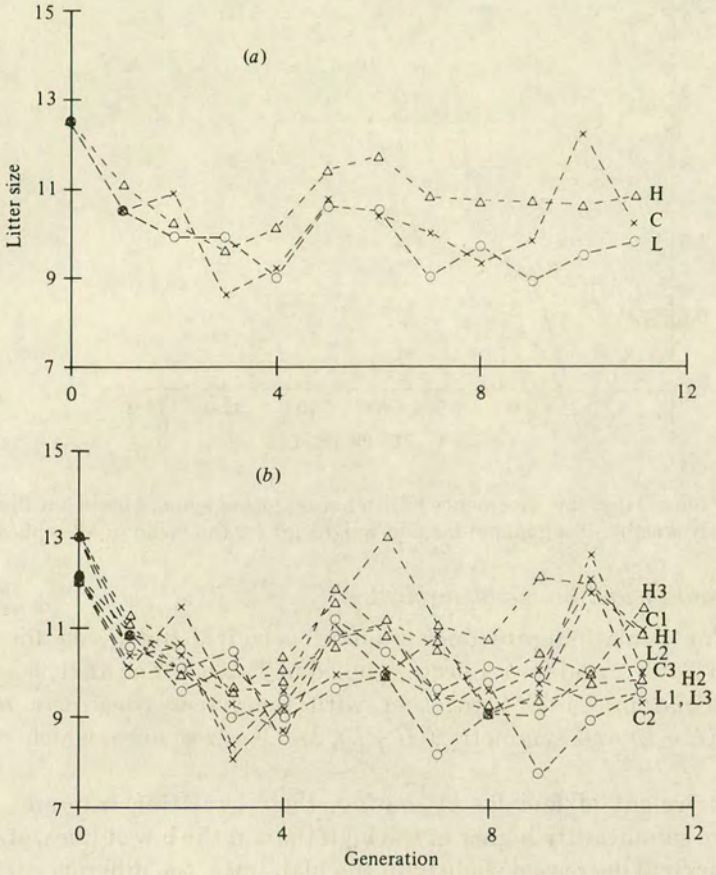

Fig. 3. *P* (protein) lines: litter size for (*a*) mean of all replicates, (*b*) individual replicates, as Fig. 1.

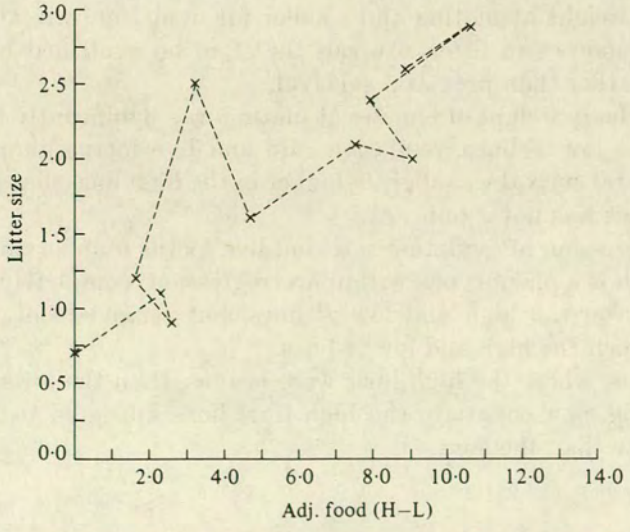

Fig. 4. *A* lines: high–low divergence of litter size plotted against high–low divergence of adjusted food intake (g) for the mean of all replicates.

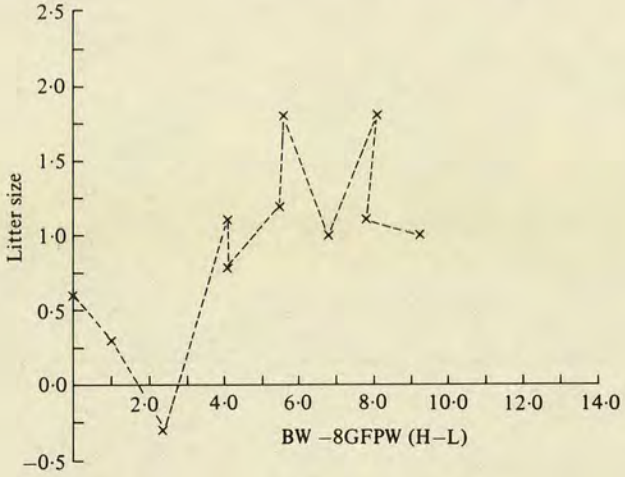

Fig. 5. *P* lines: High-low divergence of litter size plotted against high–low divergence of body weight $-8 \times$ gonadal fat pad weight (g) for the mean of all replicates.

(ii) *Ovulation rate and pre-natal survival*

Results for ovulation rate and pre-natal survival are given for individual replicates in Table 2 and for replicates pooled in Table 3. The analyses of variance are summarized in Table 4 together with the linear constrasts to estimate divergence $(H-L)$ and symmetry $((H+L)/2-C)$ of response, which were almost orthogonal.

The body weight of females at mating, their ovulation rate and live foetus number were significantly higher in the high than in the low *A* lines, and although pre-natal survival decreased slightly in the high lines, the difference from the low lines was not significant. A similar situation was observed for the *P* lines, except that relative to the *A* lines, the differences between the high and low lines were larger for body weight at mating and smaller for ovulation rate and live foetus number. The responses in litter size can therefore be explained by changes in ovulation rate rather than pre-natal survival.

Although the body weight of females at mating was significantly heavier in the high than in the low *F* lines, ovulation rate and live foetus number were not different. Pre-natal surival was slightly higher in the high lines, but the difference from the low lines was not significant.

The linear regression of ovulation rate and live foetus number on body weight at mating (which is a phenotypic, within line regression) completely removed the differences between the high and low *P* lines, but removed only some of the differences between the high and low *A* lines.

For the *F* lines, where the high lines were heavier than the lows, fitting body weight at mating as a covariate the high (fat) lines appeared to have a lower reproductive rate than the lows.

Table 2. *Means for body weight at mating (g) (B.W.), ovulation rate (O.R.), live foetus number (L.F.) and pre-natal survival % (P.S.) (numbers of mice for each mean varied from 14 to 24)*

	Replicate 1				Replicate 2				Replicate 3			
Lines	B.W.	O.R.	L.F.	P.S.	B.W.	O.R.	L.F.	P.S.	B.W.	O.R.	L.F.	P.S.
					A (adjusted food intake)							
High	28·3	16·9	12·8	80·3	24·6	12·8	11·2	86·8	30·4	17·1	13·4	79·2
Control	24·1	11·9	8·8	73·4	26·1	13·3	11·1	84·0	25·2	11·2	9·4	82·1
Low	24·5	11·9	10·2	86·6	26·8	13·7	11·6	85·4	25·0	12·2	10·1	83·2
					A (adjusted food intake)							
High*	—	—	—	—	30·8	15·8	11·5	73·5	—	—	—	—
Control*	—	—	—	—	24·2	11·9	9·7	81·8	—	—	—	—
Low*	—	—	—	—	24·7	9·6	7·5	78·8	—	—	—	—
					F (gonadal fat pad wt/body wt)							
High	27·3	12·4	9·6	78·0	27·9	16·2	13·3	83·1	23·4	11·6	10·0	87·5
Control	26·0	13·2	10·0	76·7	27·6	15·5	12·3	79·4	24·2	11·9	10·0	84·8
Low	23·1	14·3	10·3	75·1	24·2	14·2	11·4	80·0	22·1	11·7	9·4	81·9
					P (body wt − 8 × gonadal fat pad wt)							
High	29·2	14·8	11·6	78·1	28·3	13·4	11·0	82·5	30·4	15·7	12·6	80·8
Control	20·7	10·8	8·9	82·8	25·0	12·6	10·2	80·8	24·9	12·8	9·9	78·1
Low	21·3	11·3	9·5	84·8	22·3	12·1	10·6	87·5	21·5	11·2	9·2	82·9

* Repeat study.

Table 3. *Numbers of mice mated and means of body weight (g) (B.W.), ovulation rate (O.R.), live foetus number (L.F.) and pre-natal survival % (P.S.) (replicates pooled)*

Lines	No. of mice mated	No. of pregnant mice	No. of non-pregnant mice*	Means B.W. (g)	O.R.	L.F.	P.S. (%)
			A (adjusted food intake)				
High	71	70	1	28·5	15·6	12·2	79·9
Control	74	73	1	24·9	12·0	9·7	80·3
Low	77	77	0	25·2	11·8	9·8	83·5
			F (gonadal fat pad wt/body wt)				
High	62	61	1	26·2	13·4	11·0	82·9
Control	55	51	4	25·9	13·5	10·8	80·3
Low	54	54	0	23·1	13·4	10·4	79·0
			P (body wt − 8 × gonadal fat pad wt)				
High	63	62	1	29·3	14·6	11·7	80·5
Control	59	54	5	23·5	12·1	9·7	80·6
Low	55	54	1	21·7	11·5	9·8	85·1
S.E.†				0·70	0·72	0·53	2·93

* Non-pregnant mice are not included in the analyses.

† Standard errors based on between-line variance (except for pre-natal survival, where it was based on the combined variance of between-lines and between-full-sib family effect).

Repeat sampling of replicate 2 of the A lines

Some circumstantial evidence that the original sampling was unrepresentative comes from comparisons between body weights at mating of 8-week old females in the sample originally dissected, 24·6, 26·1 and 26·8 g for *H*, *C* and *L*, respectively, and body weights of 6-week old females in the selection experiment, 25·7, 22·2 and 24·8 g respectively. Likewise, live foetus numbers in the samples were 11·2, 11·1 and 11·6 respectively and 12·4, 11·1 and 9·7 live young in the selection lines. The high *A* line in replicate 2 is the only one out of the 27 lines where the 8-week weights of the sample were lower than the 6-week weights from the selection experiment. Assuming a phenotypic regression of at least +0·4 eggs per gram increase in body weight at mating (Land, 1970; table 4), it is not surprising that ovulation rate and live foetus numbers were slightly lower in the original sample of the high than the control or low line samples of replicate 2.

The results in the repeat sampling of this replicate were quite different from those obtained previously in both body weight and reproductive performance (Table 2), and were more comparable to the results of the selection experiment (Fig. 1, table 1).

4. DISCUSSION

Our results show that changes in ovulation rate, rather than pre-natal survival, are responsible for the changes in litter size in the lines selected for appetite and total lean mass. In contrast, mice selected for percentage fat do not display significant changes in litter size or ovulation rate.

The index used as the selection criterion in the total lean mass lines (body

Table 4. *Linear contrasts for differences between high and low selected lines (H–L) and symmetry, ((H+L)/2-C) and analyses of variance for body weight (g) (B.W.), ovulation rate (O.R.), live foetus number (L.F.) and pre-natal survival (%) (P.S.), before and after fitting regressions on body weight or ovulation rate*

		No. regressions fitted				Regressions fitted		
						B.W. Fitted		O.R. Fitted
Contrast	d.f.	B.W. (g)	O.R.	L.F.	P.S. (%)	O.R.	L.F.	P.S. (%)
		Contrasts				Contrasts		
A H–L	1	+3·3**	+3·8**	+2·4**	−3·6	+2·1**	+1·6*	+2·2
A Symmetry	1	+1·4*	+1·1*	+0·9**	+1·0	+0·4	+0·5	+2·7
F H–L	1	+3·1**	0·0	+0·6	+3·9	−1·6	−0·2	+3·9
F Symmetry	1	−1·3	−0·2	−0·1	+0·7	+0·5	+0·2	+0·4
P H–L	1	+7·6**	+3·1**	+2·0*	−4·6	−0·8	+0·1	+0·1
P Symmetry	1	+2·0*	+1·0	+1·1	+2·2	0·0	+0·6	+3·8
						Regression coefficients (±S.E.)		
Covariate								
B.W.	1	—	—	—	—	0·49**±0·061	+0·41**±0·072	—
O.R.	1	—	—	—	—	—	—	−1·3**±0·37
		Mean squares				Mean squares		
Replicates	6	77·77*	67·72*	39·99	357	23·54	23·00	300
Lines	12	24·11	22·54*	13·73	143	14·46**	10·57	201
Families	144	13·28**	9·85**	9·62**	447**	6·23**	9·71**	416**
Individuals								
No regressions	382	3·11	5·13	6·62	284	—	—	—
Regressions fitted	381	—	—	—	—	4·38	6·11	276

*$P < 0·05$, **$P < 0·01$, otherwise $P > 0·05$.

Tests: Contrasts, main effects and (pooled) replicates against (pooled) lines, (pooled) lines against families and families against individuals.

weight–8 × gonadal fat pad weight) has a very high correlation with body weight and the correlated changes in litter size agree in magnitude with those reported in selection studies on body weight or body weight gain (MacArthur, 1949; Falconer, 1953; Fowler & Edwards, 1960; Rahnefeld *et al.* 1966; McCarthy, 1982), as do the changes in ovulation rate (MacArthur, 1944; Fowler & Edwards, 1960; Land, 1970).

However, the correlated changes in ovulation rates from selection for appetite are larger than can be explained simply as a consequence of increases in body weight: For every gram increase in body weight at mating there is an increase of 1·15 corpora lutea in the *A* lines but an increase of only 0·41 in the *P* lines. The significant asymmetry in body weight, ovulation rate and live foetus number of the *A* lines could be real, but may have been partly due to the relatively low performance of the control mice within the sample dissected, compared to control mice used in other generations.

Fowler & Edwards (1960) have suggested from indirect evidence that ovulation rate in the mouse may be correlated more with body protein weight rather than total body weight. Sharp *et al.* (1984) found that mice from the high *A* line have become leaner than control mice, but these relatively small differences in carcass composition would only be enough to explain a small part of the higher ovulation rates observed. Further, the *F* lines with substantially changed composition and significant changes in body weight have shown little change in reproductive performance. So, what could be causing the high correlated responses in reproduction within the *A* lines? The following explanations are offered as possibilities:

(1) A major gene or genes with large effects on ovulation rate could have been present in the base population, as suggested by the early rapid response in litter size (Figs. 1 and 4). The evidence for this is, however, unconvincing. The variance of litter size within lines did not show a decline after the first few generations as would be expected following fixation of a major gene. In the study of ovulation rate and pre-natal survival, however, a large variance relative to other lines was noticed for ovulation rate in replicate 1 of the high *A* line, but this was not consistent for litter size over many generations.

(2) The high *A* line mice may ovulate more eggs in response to the dynamic effect of consuming relatively large amounts of food ('flushing').

(3) Mice are measured for food intake from 4 to 6 weeks of age. This period encompasses the onset and attainment of puberty, a process which may be physiologically associated with the determination of ovulation rate, general metabolism and of appetite. It is possible that selection for high appetite produced mice which reach their 'peak' of reproductive potential earlier in life than lines selected for body weight, or components thereof.

(4) There may be some pleiotropy between genes controlling food intake and metabolic rate and those controlling ovulation rate. We have evidence of differences in metabolic rate between the high and low appetite selections (S. Bishop, unpublished; M. Nielsen, unpublished).

Interestingly, the increases in ovulation rate in the high A lines is reflected in larger litter sizes, and has not led to a significant decline in pre-natal survival. A decline in pre-natal survival with increasing ovulation rate has been noted in previous studies (e.g. Bowman & Roberts, 1958; Fowler & Edwards, 1960). Our

results can be contrasted with the effects of direct selection where, although ovulation rate has been increased, litter size remained unchanged (Land & Falconer, 1969; Bradford, 1969).

In conclusion, directional selection for appetite and total lean mass in mice has resulted in changes in litter size and ovulation rate in the same direction as selection, those selected for appetite showing the larger responses. Associated changes in body weight can explain the differences in ovulation rate and litter size in the lean mass lines, but can only partly explain the differences in the appetite lines. Lines selected for percentage fat showed no correlated response in litter size or ovulation rate. The reasons for the large responses in ovulation rate within the appetite lines obviously need closer study.

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